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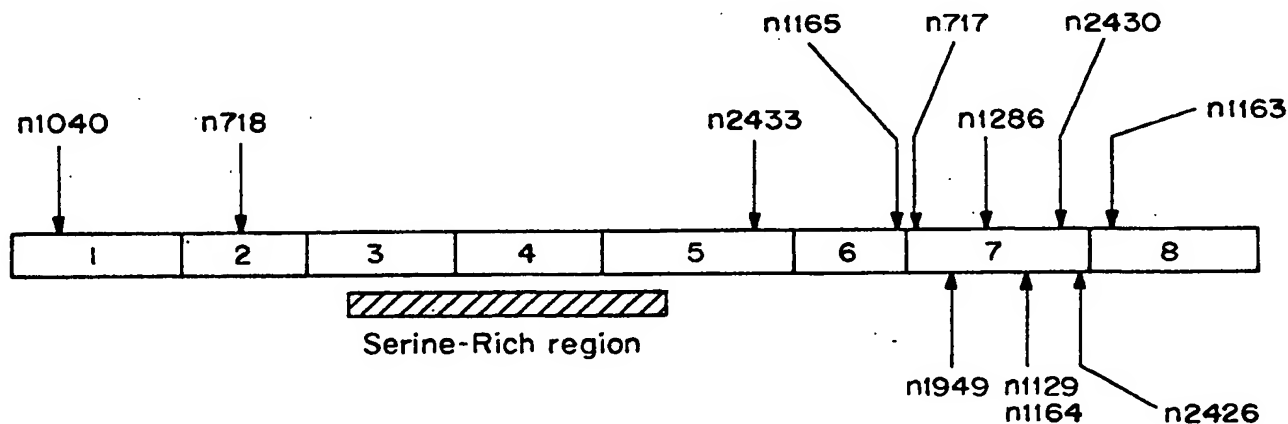
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(54) Title: INHIBITORS OF CED-3 AND RELATED PROTEINS



## (57) Abstract

Described herein is the discovery that human interleukin-1 $\beta$  convertase (ICE) is structurally similar to the protein encoded by the *C. elegans* cell death gene, *ced-3*. Comparative and mutational analyses of the two proteins, together with previous observations, suggest that the Ced-3 protein may be a cysteine protease like ICE and that ICE may be a human equivalent of the nematode cell death gene. Another mammalian protein, the murine NEDD-2 protein, was also found to be similar to Ced-3. The NEDD-2 gene is implicated in the development of the murine central nervous system. On the basis of these findings, novel drugs for enhancing or inhibiting the activity of ICE, *ced-3*, or related genes are provided. Such drugs may be useful for treating inflammatory diseases and/or diseases characterized by cell deaths, as well as cancers, autoimmune disorders, infections, and hair growth and hair loss. Furthermore, such drugs may be useful for controlling pests, parasites and genetically engineered organisms. Furthermore, novel inhibitors of the activity of *ced-3*, ICE and related genes are described which comprise portions of the genes or their encoded products.

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INHIBITORS OF CED-3 AND RELATED PROTEINSBackground

Cell death is a fundamental aspect of animal development. Many cells die during the normal development of both vertebrates (Glucksmann, *Biol. Rev. Cambridge Philos. Soc.* 26:59-86 (1951)) and invertebrates (Truman, *Ann. Rev. Neurosci.* 7:171-188 (1984)). These deaths appear to function in morphogenesis, metamorphosis and tissue homeostasis, as well as in the generation of neuronal specificity and sexual dimorphism (reviewed by Ellis et al., *Ann. Rev. Cell Biol.* 7:663-698 (1991)). An understanding of the mechanisms that cause cells to die and that specify which cells are to live and which cells are to die is essential for an understanding of animal development.

The nematode *Caenorhabditis elegans* is an appropriate organism for analyzing naturally-occurring or programmed cell death (Horvitz et al., *Neurosci. Comment.* 1:56-65 (1982)). The generation of the 959 somatic cells of the adult *C. elegans* hermaphrodite is accompanied by the generation and subsequent deaths of an additional 131 cells (Sulston and Horvitz, *Dev. Biol.* 82:110-156 (1977); Sulston et al., *Dev. Biol.* 100:64-119 (1982)). The morphology of cells undergoing programmed cell death in *C. elegans* has been described at both the light and electron microscopic levels (Sulston and Horvitz, *Dev. Biol.* 82:100-156 (1977); Robertson and Thomson, *J. Embryol. Exp. Morph.* 67:89-100 (1982)).

Many genes that affect *C. elegans* programmed cell death have been identified (reviewed by Ellis et al., *Ann. Rev. Cell Biol.* 7:663-698 (1991)). The activities of two of these genes, *ced-3* and *ced-4*, are required for the onset of almost all *C. elegans* programmed cell deaths (Ellis and Horvitz, *Cell* 44:817-829 (1986)).



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When the activity of either *ced-3* or *ced-4* is eliminated, cells that would normally die instead survive and can differentiate into recognizable cell types and even function (Ellis and Horvitz, *Cell* 44:817-829 (1986); Avery and Horvitz, *Cell* 51:1071-1078 (1987); White et al., *Phil. Trans. R. Soc. Lond. B.* 331:263-271 (1991)). Genetic mosaic analyses have indicated that the *ced-3* and *ced-4* genes most likely act in a cell autonomous manner within dying cells, suggesting that the products of these genes are expressed within dying cells and either are cytotoxic molecules or control the activities of cytotoxic molecules (Yuan and Horvitz, *Dev. Biol.* 138:33-41 (1990)).

#### Summary of the Invention

This invention is based mainly on two experimental findings and their implications: 1) that human interleukin-1 $\beta$  convertase (ICE), which converts pro-interleukin-1 $\beta$  to the active cytokine and is involved in the inflammatory response in humans, has considerable similarity to the protein encoded by the *C. elegans* cell death gene, *ced-3*; and 2) that fusion constructs containing amino-terminal portions of the *ced-3* gene can prevent cell death in *C. elegans*. As discovered by Applicant, the human ICE and nematode Ced-3 proteins have an overall amino acid identity of 28%. A higher degree of similarity was found in the carboxyl-terminal region, a region shown to be critical for the activities of both proteins. Furthermore, three sequences important for ICE activity, the region surrounding the active cysteine and two autocleavage sites, have been shown to be conserved in the *ced-3* gene product.

Thus, significant structural similarity has been shown between two proteins which previously were thought to be unrelated (to have dissimilar physiological

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roles). This finding leads to several implications, some of which are:

1) that the human ICE gene has an activity similar to that of *ced-3* in causing cell death;

5       2) that the Ced-3 protein is also a cysteine protease with a substrate specificity similar to that of ICE;

3) that mutations in the ICE gene corresponding to mutations in the *ced-3* gene will produce similar  
10 effects, such as inactivation and constitutive activation;

4) that the *ced-3* and ICE genes are members of a family of structurally related genes, referred to herein as the *ced-3*/ICE family, some of which are likely to be  
15 cell death genes and some of which may encode substrate-specific proteases;

5) that inhibitors of ICE, such as peptide aldehydes which contain the ICE recognition site or a substituted recognition site and the cowpox virus CrmA  
20 protein, may also be useful for inhibiting cell deaths; and

6) that inhibitors of *ced-3*, such as inhibitory portions of the gene or encoded product, may also be useful for inhibiting inflammation.

25       This hitherto unknown connection between a cell death protein and a protease involved in the inflammatory response provides a basis for developing novel drugs and methods for the treatment of acute and chronic inflammatory disease, of leukemias in which IL-  
30  $1\beta$  is implicated, and of diseases and conditions characterized by cell deaths (such as myocardial infarction, stroke, traumatic brain injury, viral and other types of pathogenic infection, neural and muscular degenerative diseases, aging, hair loss). In addition,  
35 drugs which increase cell deaths and which are useful

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for reducing the size or proliferative capacity of cell populations, such as cancerous cells, infected cells, cells which produce autoreactive antibodies, and hair follicle cells, as well as drugs which incapacitate or  
5 kill organisms, such as pests, parasites and recombinant organisms, can be developed using the *ced-3*, ICE, and other *ced-3*/ICE genes and their gene products.

This work also provides probes and methods for identifying additional members of the *ced-3*/ICE gene  
10 family. Genes related to *ced-3* and ICE are expected to exist in various organisms. Some of these may be cell death genes and/or proteases. The sequences of these related genes and their encoded products can be compared, for instance, using computer-based analysis,  
15 to determine their similarities. Structural comparisons, for example, would indicate those regions or features of the genes or encoded products which are likely to be functionally similar and important. Such information can be used to design drugs which mimic or  
20 alter the activity of the *ced-3*, ICE, or other *ced-3*/ICE genes, and which may, thus, be useful in the various medical and agricultural applications mentioned above.

In addition, another mammalian protein, the murine NEDD-2 protein, was also found to be similar to Ced-3.  
25 Interestingly, NEDD-2 is not significantly similar to ICE. Thus, another potential mammalian cell death gene was identified.

Also described herein is the discovery that fusion constructs which encode an amino-terminal portion of the  
30 Ced-3 protein fused to  $\beta$ -galactosidase act as inhibitors of cell death in *C. elegans*. Due to its structural similarity to Ced-3, constructs encoding corresponding portions of the human ICE protein are also expected to inhibit the enzymatic activity of ICE in cleaving  
35 interleukin- $1\beta$ . Thus, inhibitors comprising an amino-

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terminal portion of the Ced-3 protein, ICE protein or another member of the Ced-3/ICE family and RNAs and DNA constructs which express these protein portions are potentially useful for decreasing cell deaths and/or inflammation involved in various pathologies. Methods for identifying other inhibitory portions of the *ced-3* and ICE genes are also described.

Furthermore, deletion of the inhibitory amino-terminal portions of the *ced-3* and ICE genes may result in constitutive activation of the genes. Constitutively activated carboxyl-terminal portions of the genes, or their encoded products, may thus be useful in applications where increased cell deaths or an increased inflammatory response are desired.

#### 15 Brief Description of the Drawings

Figure 1 shows the physical and genetic maps of the *ced-3* region on chromosome IV.

Figure 2 summarizes the experiments to localize *ced-3* within C48D1. Restriction sites of plasmid C48D1 and subclone plasmids are shown. Ced-3 activity was scored as the number of cell corpses in the head of L1 young animals. ++, the number of cell corpses above 10. +, the number of cell corpses below 10 but above 2. -, the number of cell corpses below 2.

25 Figures 3A-H show the nucleotide sequence (SEQ ID NO:1) of *ced-3* and deduced amino acid sequence (SEQ ID NO:2). The genomic sequence of the *ced-3* region was obtained from plasmid pJ107. The introns and the positions of 12 *ced-3* mutations are indicated. The likely translation initiation site is indicated by a solid arrowhead. The SL1 splice acceptor of the RNA is boxed. Repetitive elements are indicated as arrows above the relevant sequences. Numbers on the sides

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indicate nucleotide positions. Numbers under the amino acid sequence indicate codon positions.

Figure 4A shows the genomic structure of the *ced-3* gene and the location of the mutations. The sizes of the introns and exons are given in bp. The downward arrows indicate the positions of 12 EMS-induced mutations of *ced-3*. The arrow pointing right indicates the direction of transcription. The solid arrowhead indicates the translation initiation site. The open arrowhead indicates the termination codon.

Figure 4B shows the locations of the mutations relative to the exons (numbered 1-7) and the encoded serine-rich region in *ced-3*.

Figure 5 is a Kyte-Doolittle hydrophobicity plot of the Ced-3 protein.

Figures 6A-B show the alignment of the amino acid sequences of Ced-3 (SEQ ID NO:2) and human interleukin-1 $\beta$  convertase (ICE; SEQ ID NO:4). Vertical bars indicate identical amino acids and single and double dots indicate similar amino acids, where double dots signifies closer similarity than a single dot. The serine-rich region and inactivating mutations of Ced-3 are indicated. The active site and autocleavage sites of ICE are indicated. The portions of the Ced-3 protein encoded by the BGAFQ and PBA constructs are also shown.

Figure 7 shows the alignment of the amino acid sequences of Ced-3 (SEQ ID NO:2) and murine NEDD-2 (SEQ ID NO:13). Vertical bars and single and double dots signify degrees of similarity as in Figures 6A-B. Inactivating mutations of Ced-3 are shown.

Figure 8A shows the alignment of the amino-terminal regions of the Ced-3 proteins of three nematode species (*C. briggsae*, *C. elegans*, and *C. vulgaris*) and mouse (SEQ ID NO:14) and human ICEs. A consensus sequence is also shown. Amino acid positions with the same residue

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in more than half of the sequences are shaded.  
Completely conserved amino acids are also boxed.

Figure 8B shows the alignment of carboxyl-terminal regions of the three nematode Ced-3 proteins, human and 5 mouse ICEs, and the mouse NEDD-2 protein. Except for NEDD-2, these sequences are contiguous with the corresponding sequences shown in Figure 8A. A consensus sequence and amino acid conservation are also shown.

Figure 9 shows a comparison of the Ced-3 proteins 10 of *C. elegans* (line 1; SEQ ID NO:2) and two related nematode species, *C. briggsae* (line 2; SEQ ID NO:5) and *C. vulgaris* (line 3; SEQ ID NO:6). The conserved amino acids are indicated by ".". Gaps inserted in the sequence for the purpose of alignment are indicated by 15 "\_".

Figure 10 is the interleukin-1 $\beta$  convertase cDNA sequence (SEQ ID NO:3).

Figure 11A is a schematic representation of two fusion constructs that can prevent programmed cell 20 death.

Figure 11B is a schematic representation of the lacZ-containing portion of the fusion constructs.

#### Detailed Description of the Invention

This invention is based on the discovery that the 25 human enzyme interleukin-1 $\beta$  convertase (ICE) has significant structural similarity to the protein product of the *C. elegans* cell death gene, *ced-3*. The activities of *ced-3* and another cell death gene, *ced-4*, have been shown to be required for almost all the cell 30 deaths which occur during the development of the nematode. ICE is a cysteine protease whose physiological significance has been thought to be related to its role in the maturation of one form of interleukin-1 (IL-1), a major mediator of the immune and

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inflammatory response (Fuhlbrigge et al., in: *The Year in Immunology*, Cruse and Lewis (eds.), Karger, Basel, 1989, pp. 21-37). There are two distantly related forms of IL-1,  $\alpha$  and  $\beta$ , of which the  $\beta$  form is the predominant species. ICE selectively converts pro-interleukin-1 $\beta$  to the active cytokine, IL-1 $\beta$ . The production of active IL-1 $\beta$  has been implicated in acute and chronic inflammatory diseases, septic shock, and other physiological processes, including wound healing and resistance to viral infection (Ray et al., *Cell* 69:597-604 (1992)). As a result of this discovery, an enzyme which has been known to be involved in the inflammatory response and inflammatory diseases is implicated as having a role in cell death processes. This discovery is consistent with the notion that cell death genes equivalent to the nematode *ced-3* gene function in a variety of organisms. The structural similarity between their gene products suggests that the ICE gene is a human equivalent of the *ced-3* cell death gene. As further described below, the conservation of certain features of ICE in the Ced-3 protein further suggests that Ced-3 is a protease with a substrate-specificity similar to that of ICE.

Furthermore, the identification of *ced-3* and ICE as structurally related genes (i.e., genes whose encoded products, or which themselves, are structurally similar) presents the possibility that a family of structurally related genes exists and provides probes to identify additional members of this *ced-3*/ICE gene family. Comparison of the genes within this family could indicate functionally important features of the genes or their gene products, and thus, provide information for designing drugs which are useful for treating conditions characterized by cell deaths and/or inflammatory disease.

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This discovery provides novel drugs based on the *ced-3*, ICE and other *ced-3*/ICE genes and encoded products that inhibit the production of IL-1 $\beta$  and are useful for treatment (preventive and therapeutic) of acute and chronic inflammatory disease, as well as drugs which reduce cell deaths and are useful for treatment of diseases and conditions involving cell deaths (such as myocardial infarction, stroke, traumatic brain injury, viral and other types of pathogenic infection, degenerative diseases, aging, and hair loss). These drugs may also be useful for treating leukemias in which IL-1 $\beta$  has been implicated.

Drugs or agents which increase cell deaths can also be developed based on the *ced-3*, ICE, and related genes and gene products; such drugs or agents may be useful for killing or incapacitating undesired cell populations (such as cancerous cells, infected cells, cells which produce autoreactive antibodies and hair follicle cells) or undesired organisms (such as pests, parasites, and genetically engineered organisms). Drugs are also provided which increase IL-1 $\beta$  production and, therefore, the inflammatory and immune response. These drugs may be helpful for providing increased resistance to viral and other types of infection.

Also described herein is the discovery that fusion constructs containing amino-terminal portions of the *ced-3* gene can inhibit the activity of the intact gene when expressed in otherwise wild-type worms. Due to the similarity between ICE and *Ced-3*, it is likely that the corresponding amino-terminal portions of the ICE gene will also inhibit the enzymatic activity of ICE in cleaving interleukin-1 $\beta$ . Thus, novel inhibitors of the *ced-3* and ICE genes are provided which may be useful for decreasing cell deaths and/or inflammation involved in various pathologies.



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This work has also shown that Ced-3 and the murine NEDD-2 protein are structurally similar. Thus, drugs for increasing or decreasing cell deaths can be developed based on the NEDD-2 gene and its encoded  
5 products.

The above-described discoveries, and their implications, and novel drugs and treatments for diseases related to cell death and/or inflammation arising therefrom are described in further detail below.

10 As used herein, the activity of a gene is intended to include the activity of the gene itself and of the encoded products of the gene. Thus, drugs and mutations which affect the activity of a gene include those which affect the expression as well as the function of the  
15 encoded RNA and protein. The drugs may interact with the gene or with the RNA or protein encoded by the gene, or may exert their effect more indirectly.

#### The *ced-3* Gene

The *C. elegans ced-3* gene was cloned by mapping DNA  
20 restriction fragment length polymorphisms (RFLPs) and chromosome walking (Example 1; Figure 1). The gene was localized to a 7.5 kb fragment of cloned genomic DNA by complementation of the *ced-3* mutant phenotype (Figure 2). A 2.8 kb transcript was further identified. The  
25 *ced-3* transcript was found to be most abundant in embryos, but was also detected in larvae and young adults, suggesting that *ced-3* is expressed not only in cells undergoing programmed cell death.

A 2.5 kb cDNA corresponding to the *ced-3* mRNA was  
30 sequenced. The genomic sequence cloned in the plasmid pJ107 was also determined (Figure 3; SEQ ID NO:1). A comparison with the cDNA sequence revealed that the *ced-3* gene has 7 introns which range in size from 54 to 1195 bp (Figure 4A). The four largest introns, as well as

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sequences 5' of the start codon, contain repetitive elements (Figure 3), some of which have been previously characterized in non-coding regions of other *C. elegans* genes such as *fem-1* (Spence et al., *Cell* 60:981-990 (1990)), *lin-12* (J. Yochem, personal communication), and *myoD* (Krause et al., *Cell* 63:907-919 (1990)). The transcriptional start site was also mapped (Figure 3), and a *ced-3* transcript was found to be trans-spliced to a *C. elegans* splice leader, SL1.

10 Twelve EMS-induced *ced-3* alleles were also sequenced. Eight of the mutations are missense mutations, three are nonsense mutations, and one is a putative splicing mutation (Table 1). This identification of *ced-3* null alleles, together with  
15 results of genetic analysis of nematodes homozygous for these null mutations in *ced-3*, indicate that, like *ced-4*, *ced-3* function is not essential to viability. In addition, 10 out of the 12 mutations are clustered in the carboxyl-terminal region of the gene (exons 6-8,  
20 Figure 4B), suggesting that this portion of the encoded protein may be important for activity.

The *ced-3* gene encodes a putative protein of 503 amino acids (Figure 3; SEQ ID NO:2). The protein is very hydrophilic and no significantly hydrophobic region  
25 can be found that might be a transmembrane domain (Figure 5). One region of the Ced-3 protein is very rich in serine (Figures 6A-B). Comparison of the *C. elegans* protein with the Ced-3 proteins of two related nematodes species, *C. briggsae* and *C. vulgaris*, shows  
30 conservation of the serine-rich feature without conservation of the amino acid sequence in this region (Figure 9; SEQ ID NO:5-6). This suggests that the exact sequence of this serine-rich region may not be important but that the serine-rich feature is. This hypothesis is  
35 supported by analysis of *ced-3* mutations: none of 12

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EMS-induced *ced-3* mutations is in the serine-rich region (Figure 4B). It is possible that the serine-rich region in *Ced-3* is another example of semi-specific protein-protein interaction, similar to acid blobs in

5 transcription factors and basic residues in nuclear localization signals. In all these cases, the exact primary sequence is not important.

The serine-rich region may function as a site for post-translational regulation of *Ced-3* activity through

10 protein phosphorylation of the serine residues by a Ser/Thr kinase. McConkey et al. (*J. Immunol.* 145:1227-1230 (1990)) have shown that phorbol esters, which stimulate protein kinase C, can block the death of cultured thymocytes induced by exposure to  $Ca^{++}$

15 ionophores or glucocorticoids (Wyllie, *Nature* 284:555-556 (1980); Wyllie et al., *J. Path.* 142:67-77 (1984)). It is possible that protein kinase C may inactivate certain cell death proteins by phosphorylation and, thus, inhibit cell death and promote cell proliferation.

20 Several agents that can elevate cytosolic cAMP levels have been shown to induce thymocyte death, suggesting that protein kinase A may also play a role in mediating thymocyte death. Further evidence suggests that abnormal phosphorylation may play a role in the

25 pathogenesis of certain cell-degenerative diseases. For example, abnormal phosphorylation of the microtubule-associated protein Tau is found in the brains of Alzheimer's disease and Down's syndrome patients (Grundke-Iqbal et al., *Proc. Natl. Acad. Sci. USA*

30 83:4913-4917 (1986); Flament et al., *Brain Res.* 516:15-19 (1990)). Thus, it is possible that phosphorylation may have a role in regulating programmed cell death in *C. elegans*. This is consistent with the fairly high levels of *ced-3* and *ced-4* transcripts which suggest that

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transcriptional regulation alone may be insufficient to regulate programmed cell death.

Structural Relatedness of the ced-3 and Human Interleukin-1 $\beta$  Convertase Genes and Functional

5 Implications

A search of GenBank, PIR and SWISS-PROT databases using the Blast program (National Center for Biotechnology Information) revealed that human interleukin-1 $\beta$  convertase (ICE) has a 28% amino acid  
10 identity with the Ced-3 protein (Figures 6A-B). A comparable level of overall similarity was found between ICE and the Ced-3 proteins from two other nematode species, *C. briggsae* and *C. vulgaris*.

The carboxyl-terminal regions of Ced-3 and ICE  
15 (amino acids 250-503 and amino acids 166-404, respectively) were found to be more conserved (33% identity) than the amino-terminal portions of the two proteins (22% identity). A comparison of human and murine ICEs also indicated a high degree of similarity  
20 (80% identity) in the carboxyl-terminal region compared with an overall identity of 62% (Cerretti et al., Science 256:97-100 (1992)). Furthermore, deletion analysis of the ICE cDNA sequence has shown that the amino-terminal 119 amino acids of ICE are not required  
25 for enzymatic activity, but that deletions of the carboxyl-terminal region eliminate the enzyme's ability to process pro-IL-1 $\beta$  (Cerretti et al., 1992 supra). The observation that most of the inactivating mutations of *ced-3* cluster in the carboxyl-terminal region (Figure  
30 4B) suggests that the activity of Ced-3 also resides (at least partially) in this region. Thus, the identification of the carboxyl-terminal regions of the two proteins as functional domains and the marked similarity of these regions suggest that the Ced-3 and

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ICE proteins have similar activities, i.e., that ICE has cell death activity similar to Ced-3 and Ced-3 has protease activity similar to ICE.

The possibility that Ced-3 has protease activity is further supported by the observation that the region surrounding the active cysteine and two autocleavage sites of ICE appear to be conserved in the Ced-3 protein. As shown in Figures 6A-B, the five amino acids (QACRG, amino acids 283 to 287) surrounding the active cysteine of ICE (Thornberry et al., *Nature* 356:768-774 (1992)) are conserved in amino acids 356 to 360 of Ced-3; this pentapeptide is the longest conserved sequence between ICE and Ced-3. This peptide is also conserved in the Ced-3 proteins of *C. briggsae* and *C. vulgaris* (Figure 9). One inactivating mutation of *ced-3*, *n2433*, introduces a glycine to serine change near the putative active cysteine (Figures 6A-B). The human ICE gene encodes a precursor enzyme which is autoproteolytically cleaved at two major sites (amino acids 103 and 297) by the active form of the enzyme (Thornberry et al., 1992 *supra*). The Asp-Ser dipeptides of both autocleavage sites are conserved in Ced-3 (at amino acids 131 and 371) (Figures 6A-B). The conservation of these functionally important sequences strongly suggests that, like ICE, Ced-3 is a cysteine protease with a similar substrate-specificity. Ced-3 would, therefore, be expected to cleave the IL-1 $\beta$  precursor, as well as other substrates of ICE.

The possibility that ICE is a cell death gene is consistent with evidence which suggests that the production of active IL-1 $\beta$  is involved with cell death processes. Firstly, a variety of studies has suggested that IL-1 $\beta$  can prevent cell death (McConkey et al., *J. Biol. Chem.* 265:3009-3011 (1990); Mangan et al., *J. Immun.* 146:1541-1546 (1991); Sakai et al., *J. Exp. Med.*

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- 166:1597-1602 (1987); Cozzolino et al., *Proc. Natl. Acad. Sci. USA* 86:2369-2373 (1989)). Secondly, active, mature IL-1 $\beta$  appears to be released from cells undergoing cell death. Studies on murine macrophages
- 5 suggest that release of the active form seems not to be merely due to the lysis of the cells or leaking of cell contents. When murine peritoneal macrophages were stimulated with lipopolysaccharide (LPS) and induced to undergo cell death by exposure to extracellular ATP,
- 10 mature active IL-1 $\beta$  was released into the culture supernatant. In contrast, when the cells were injured by scraping, IL-1 $\beta$  was released exclusively as the inactive proform (Hogquist et al., *Proc. Natl. Acad. Sci. USA* 88:8485-8489 (1991)).
- 15 The similarity between ICE and Ced-3 strongly supports the hypothesis that ICE is involved in cell death. Since Ced-3 is necessary for cell death, one suggestion is that ICE is also necessary for cell death. It is possible that IL-1 $\beta$  can cause cell death.
- 20 Alternatively, ICE could produce products besides IL-1 $\beta$ , one or more of which can cause cell death. The observation that the ICE transcript is detected in cells that lack IL-1 $\beta$  expression (Cerretti et al., 1992 *supra*) supports this idea.
- 25 The finding of a human gene related to the nematode *ced-3* gene is consistent with the idea that cell death genes which are structurally related and/or functionally similar to the nematode *ced-3* gene exist in a variety of organisms. This idea is supported by evidence that cell
- 30 deaths occurring in a variety of organisms, including vertebrates and invertebrates, and possibly microbes and plants, as well as cell deaths observed in various developmental and pathologic situations share a common genetic mechanism. Evidence for this hypothesis is
- 35 discussed in Example 2. The structural relatedness of

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ICE suggests that it is a mammalian equivalent of the nematode cell death gene, *ced-3*. The cDNA sequence of ICE is shown in Figure 10 (SEQ ID NO:3).

The *ced-3*/ICE Gene Family and Uses Thereof

5       The ICE and *ced-3* genes can be used to isolate additional structurally related genes, including genes from other organisms. Such genes may be identified using probes derived from both the *ced-3* and ICE gene sequences and known techniques, including nucleic acid  
10 hybridization, polymerase chain reaction amplification of DNA, screening of cDNA or genomic libraries, and antibody screening of expression libraries. The probes can be all or portions of the genes which are specific to the genes, RNA encoded by the genes, degenerate  
15 oligonucleotides derived from the sequences of the encoded proteins, and antibodies directed against the encoded proteins. The sequences of the genes and their protein products can also be used to screen DNA and protein databases for structurally similar genes or  
20 proteins.

One strategy for detecting structurally related genes in a number of organisms is to initially probe animals which are taxonomically closely related to the source of the probes, for example, probing other worms  
25 with a *ced-3*-derived probe, or probing other mammals with an ICE-derived probe. Closely related species are more likely to possess related genes or gene products which are detected with the probe than more distantly related organisms. Sequences conserved between *ced-3* or  
30 ICE and these new genes can then be used to identify similar genes from less closely related species. Furthermore, these new genes provide additional sequences with which to probe the molecules of other animals, some of which may share conserved regions with

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the new genes or gene products but not with the original probe. This strategy of using structurally related genes in taxonomically closer organisms as stepping stones to genes in more distantly related organisms can  
5 be referred to as walking along the taxonomic tree.

Together, *ced-3*, ICE, and related genes obtained as described above would comprise a family of structurally related genes, referred to herein as the *ced-3*/ICE gene family. It is highly likely that at least some of these  
10 additional family members would exhibit cell death and/or protease activity. The new genes can be tested for protease activity using known assay methods. For example, the sequence of the protein encoded by a new gene may indicate an active site and substrate-  
15 specificity similar to that of ICE, such as observed in *Ced-3*. This activity can then be verified using the transient expression assays and purified enzyme assays previously described (Cerretti et al., *Science* 256:97-100 (1992); Thornberry et al., *Nature* 356:768-774  
20 (1992)). Cell death activity can be tested in bioassays using transgenic nematodes. A candidate cell death gene, such as the ICE gene, can be injected into *Ced-3*-deficient mutant animals to determine whether the gene complements the *ced-3* mutation. Expression libraries  
25 can also be screened for cell death genes by this assay.

The *ced-3*, ICE and other related genes which have cell death activity can be used to develop and identify drugs which reduce or increase cell deaths. Drugs which reduce cell deaths are potentially useful for treating  
30 diseases and conditions characterized by cell deaths, such as myocardial infarction, stroke, viral and other pathogenic infections (e.g., human immunodeficiency virus), traumatic brain injury, neural and muscular degenerative diseases, and aging. Drugs which cause  
35 cell deaths can be used to control or reduce undesired



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cell populations, such as neoplastic growths and other cancerous cells, infected cells, and cells which produce autoreactive antibodies. Undesired organisms, such as pests, parasites, and recombinant organisms, may also be  
5 incapacitated or killed by such drugs.

ICE has been implicated in the growth of certain leukemias (Sakai et al., *J. Exp. Med.* 166:1597 (1987); Cozzolino et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:2369 (1989); Estrov et al., *Blood* 78:1476 (1991); Bradbury et  
10 al., *Leukemia* 4:44 (1990); Delwel et al., *Blood* 74:586 (1989); Rambaldi et al., *Blood* 78:3248 (1991)). The observation that the human ICE gene maps to chromosome location 11q23, a site frequently involved in DNA rearrangements seen in human cancers (C. Cerretti et  
15 al., *Science* 256: 97-100 (1992)), further suggests that ICE is involved in cancer. The finding that ICE probably functions in cell death implies that ICE and other related genes, like *ced-3*, may be used to develop drugs to control cancerous growth.

20 In addition, since cell death plays an important role in mammalian hair growth, it seems likely that by controlling cell death, one could cause or prevent hair loss. It has been found that *bcl-2*, a human gene which is structurally related to the gene which prevents cell  
25 deaths in nematode development (*ced-9*), is expressed in the hair follicle in a cell-cycle dependent manner. *ced-9* has been shown to act by antagonizing the activities of the cell death genes, *ced-3* and *ced-4*. Together, these findings suggest that genes equivalent  
30 to the *ced-3*, *ced-4*, and *ced-9* genes are involved in the physiology of mammalian hair growth and loss.

Drugs which increase cell deaths may comprise *ced-3*, ICE, and other *ced-3*/ICE family members, their RNA and protein products, constitutively activated  
35 mutants of the genes and encoded products, and peptide

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and non-peptide mimetics of the proteins. Drugs which decrease cell deaths may comprise antisense RNA complementary to the mRNA of a cell death gene, or mutant cell death genes or encoded products, that no longer cause cell death and interfere with the function of wild-type genes. Furthermore, drugs comprising agonists and antagonists of the cell death genes can be designed or identified using the genes or their gene products as targets in bioassays. The bioassays can be conducted in wild-type, mutant, or transgenic nematodes, in which an alteration in programmed cell deaths is an indicator of an effective agonist or antagonist. Bioassays can also be performed in cultured cells transfected with the target cell death gene, into which the substance being tested is introduced. In bioassays for antagonists of cell death, the cultured cells should be put under conditions which induce the activity of the target cell death gene.

Uses of bioassays utilizing *C. elegans* are exemplified by the following:

- 1) use of normal, wild-type nematodes to screen for drugs or genes that inactivate *ced-3* and hence, prevent programmed cell deaths;
- 2) use of normal, wild-type nematodes to screen for drugs or genes that activate *ced-3* and hence, cause excess cell deaths;
- 3) use of mutant nematodes which overexpress *ced-3* or which express a constitutively activated *ced-3* gene to identify drugs or genes that prevent excess cell deaths caused by the *ced-3* mutation;
- 4) use of mutant nematodes which underexpress *ced-3* or which express an inactivated *ced-3* gene to identify drugs or genes that mimic or complement the *ced-3* mutation;

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5) use of transgenic nematodes (with an inactivated endogenous *ced-3* gene) in which either a wild-type or mutant form of ICE or other *ced-3*/ICE family member causes excess cell deaths to identify  
5 drugs or genes which antagonize the activity of the transgene; and

6) use of transgenic nematodes which carry a transgene that inhibits cell death (e.g., a transgene that expresses an inhibitory fragment of *ced-3*, ICE or  
10 related gene, as described below) to identify drugs that overcome this inhibition and cause cell death.

Drugs can be introduced into nematodes by diffusion, ingestion, microinjection, shooting with a particle gun or other methods. They can be obtained  
15 from traditional sources such as extracts (e.g., bacterial, fungal or plant) and compound libraries, or can be provided by newer methods of rationale drug design. Information on functionally important regions of the genes or gene products, gained by sequence  
20 comparisons and/or mutational analysis may provide a basis for drug design. Genes can be microinjected into nematodes to produce transgenic nematodes. Individual genes or cDNA and genomic DNA libraries can be screened in this manner.

25 Agonists and antagonists may also be derived from genes which are not cell death genes, but which interact with, regulate or bypass cell death genes. Such interacting genes may be tested by the bioassays mentioned above, as well as by *in vivo* genetics in  
30 nematodes. In this latter method, interacting genes are identified as secondary mutations which suppress or enhance the *ced-3* mutation. The sequences of these interacting genes can then be used to identify structurally related interacting genes in other  
35 organisms.

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Similarly, anti-inflammatory drugs may be developed or identified using *ced-3*, ICE and other family members and their encoded products. Drugs which enhance ICE activity may also be useful for boosting the  
5 inflammatory response to viral and other infections.

In addition, the availability of a number of structurally related genes makes it possible to carry out structural comparisons. Conserved regions or features of the genes or their encoded products are  
10 likely to be functionally significant for cell death and/or protease activity. This information could be helpful in designing or selecting drugs which would mimic or affect the activity of the genes.

Moreover, conservation of functional domains among  
15 *ced-3*/ICE family members or their encoded products suggests not only that these genes have similar activities, but that they and their encoded products function via similar mechanisms. This suggests that mutations in conserved regions, mimetics based on  
20 conserved regions, and agonists and antagonists which affect the function of conserved regions of one *ced-3*/ICE gene or encoded protein will similarly affect other genes or encoded proteins in the family. This is the rationale behind the use of Ced-3 inhibitors to  
25 inhibit ICE and inflammation, and the use of anti-inflammatory drugs which act by inhibiting ICE to inhibit the *ced-3* gene and reduce cell deaths (described further below).

Furthermore, drugs which affect the cell death  
30 and/or inflammatory activities of the *ced-3* and ICE genes may also affect other as yet undiscovered activities of these genes. The biology of IL-1 $\beta$  and ICE is only incompletely understood at the present time, and it is very likely that other functions of both IL-1 $\beta$  and  
35 ICE may be discovered. These may include new activities

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or new physiological processes or diseases in which the respective cytokinetic and proteolytic activities of these molecules are involved. In either case, drugs (such as inhibitory protein portions) which affect ICE  
5 activity are likely to affect the new activities and processes, as well.

In addition, mutations and drugs which alter or mimic the activity of one member of the *ced-3*/ICE family can be engineered based on what is known about mutations  
10 and drugs affecting another family member with which it shares a conserved region. Mutations in conserved regions which correspond to those found in another family member could be used to produce similar effects. For example, five out of nine inactivating point  
15 mutations analyzed in *ced-3* were found to result in alterations of amino acids which are conserved between ICE and *Ced-3* (Figures 6A-B). Amino acid substitutions in ICE corresponding to those in *Ced-3* are also expected to result in inactivation. The inhibitory amino-  
20 terminal gene portions and constitutively activated carboxyl-terminal gene portions described below are further examples of corresponding mutations which can be made in genes of the *ced-3*/ICE family.

Comparison of *Ced-3*, ICE, and related proteins may  
25 also provide insights into the substrate-specificity of ICE and related enzymes. Previous studies on ICE have not identified a consistent consensus cleavage site. A comparison of the *Ced-3* and ICE autocleavage sites, together with the cleavage site of pro-IL-1 $\beta$ , reveals  
30 that cleavage always occurs after an Asp residue. For this reason, it is likely that *Ced-3*, ICE, and related proteins are proteases which cleave after some aspartate residues or, perhaps at lower efficiencies, all aspartate residues.

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A further use of *ced-3*/ICE family members is to provide diagnostic probes (DNA, RNA, oligonucleotides and antibodies) for diseases involving cell deaths and inflammation in humans and other organisms. It is likely that such diseases are associated with abnormalities in *ced-3*/ICE genes and their gene products. The probes can be used to detect abnormalities in the sequence, level and/or activities of the genes and encoded RNA and protein products. The diseases may be genetic, in which case, the probes may be used in patient and pre-natal testing, or non-genetic, in which case, RNAs and proteins may be examined. In particular, the finding that ICE is a putative cell death gene makes this gene and its derivative molecules potentially useful as diagnostic probes for diseases characterized by cell deaths. Similarly, *ced-3* and its derivative molecules are potentially useful for detecting abnormalities in pathologies in which inflammation is evident. The usefulness of these probes may be multiplied as more genes with known physiological functions are found to be structurally related to *ced-3* and ICE.

#### Structural Relatedness of *ced-3* and the Murine NEDD-2 Gene

Database searches also revealed that another mammalian protein is similar to the Ced-3 protein (Figure 7). The murine NEDD-2 protein has 27% amino acid identity and 55% similarity to a carboxyl-terminal portion of Ced-3. The NEDD-2 protein is expressed in the brain of mouse embryos and much less in the murine adult brain; the protein is thought to be involved in the development of the murine central nervous system (Kumar et al., *Biochem. Biophys. Res. Comm.* 185(3):1155-1161 (1992)). The structural similarity between the

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NEDD-2 and *ced-3* gene products suggests that the NEDD-2 gene is also involved in cell death processes which occur during development, and further supports the hypothesis that genes which are structurally and  
5 functionally related to the nematode *ced-3* gene function in a variety of organisms. Interestingly, the NEDD-2 amino acid sequence is not significantly similar to that of human ICE.

The similarity of the amino acid sequences of Ced-3  
10 and NEDD-2 further suggests that mutations of the NEDD-2 gene which produce alterations in the protein corresponding to alterations in Ced-3 resulting from the mutations, *n1129*, *n1164*, *n2426* and *n1163* (see Figure 7), will inactivate the NEDD-2 gene.

15 This invention includes all and portions of the NEDD-2 gene, mutated NEDD-2 genes corresponding to known *ced-3* mutations, RNAs and proteins encoded by the wild-type and mutated genes, and mimetics and other drugs derived from these genes and gene products, which are  
20 useful for controlling cell death.

Figures 8A and 8B show alignments of the amino-terminal and carboxyl-terminal regions, respectively, of the Ced-3 proteins of the three nematode species (*C. briggsae*, *C. elegans*, and *C. vulgaris*), the human and  
25 murine ICEs and the murine NEDD-2 protein (in 8B only). As shown in these figures (boxed portions), a number of amino acids are completely conserved among these structurally related proteins, and thus, are likely to be important functionally. Mutations of these sites  
30 would be expected to alter the activity of the genes.

#### Inhibitory Portions of the *ced-3* Gene

Fusion constructs containing portions of the *ced-3* gene were found to prevent programmed cell death when expressed in wild-type *C. elegans*. These constructs are

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represented schematically in Figure 11A. The BGAFQ construct contains a portion of the *ced-3* gene fused 5' of the *E. coli lacZ* gene and another *ced-3* portion fused 3' of *lacZ*. The 5' *ced-3* portion is the genomic  
5 sequence from a *Bam*HI site located about 300 base pairs upstream of nucleotide 1 of the sequence shown in Figure 3 to a *Sal*I site at nucleotide 5850. This portion spans sequences 5' of the SL1 acceptor site (nucleotide 2161) to include the 372 codons of the amino-terminal region.  
10 The 3' *ced-3* portion of BGAFQ is the genomic sequence from a *Not*I site at nucleotide 5927 in the *ced-3* gene to an *Apa*I site located about 1.5 kb downstream of nucleotide 7653 of the sequence in Figure 3. This portion contains the carboxyl-terminal codons from 398  
15 to the end (codon 503) and 3' untranslated sequences.

The PBA construct has a smaller portion of the *ced-3* gene which is the genomic sequence from the same *Bam*HI site as in BGAFQ to a *Bgl*II site at nucleotide 3020 (Figure 11A) fused 5' of the *lacZ* gene. This *ced-3*  
20 portion spans sequences 5' of the SL1 acceptor site to include the first 149 codons of the amino-terminal region.

Both constructs were made using the pBluescript vector (Stratagene) and fragments containing the *lacZ*  
25 construct from the pPD vectors of Fire (*EMBO J.* 5:2673-2690 (1986)). The *lacZ*-containing portion has the entire *lacZ* coding sequence except for the first 11 codons. In addition, there is a synthetic intron and a nuclear localization signal upstream of the *lacZ* gene  
30 and a fragment of the 3' end of the *unc-54* gene downstream of the *lacZ* gene (Figure 11B). Construct PBA was made by inserting a *Bam*HI-*Apa*I fragment containing the *lacZ* construct shown in Figure 11B from Andy Fire's vector, pPD22.04, into the *Bgl*II-*Apa*I fragment of the  
35 *ced-3*-containing plasmid, pJ40. Construct BGAFQ was



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made by inserting a *SalI*-*EagI* fragment containing the same *lacZ* construct from pPD22.04 into the *SalI*-*NotI* fragment of pJ40A, which is pJ40 without the *NotI* site in the vector.

5        Table 2 shows the results of injecting wild-type nematodes with the two constructs. These results indicate that the BGAFQ and PBA fusion constructs prevent cell deaths which normally occur in the development of the nematodes. These fusion constructs were further  
10 observed to prevent cell deaths and the apparently associated inviability caused by a loss-of-function mutation in *ced-9*, a gene which functions to keep certain cells from dying during nematode development, and which has been shown to act by antagonizing *ced-3*  
15 and second cell death gene, *ced-4*.

Both constructs express  $\beta$ -galactosidase activity in wild-type nematodes. Since the pBluescript vector does not contain eukaryotic transcriptional or translational start sites, these signals are probably supplied by the  
20 *ced-3* gene portions fused 5' of *lacZ*. Furthermore, since the PBA construct works to prevent cell death, it seems that the *ced-3* portion in BGAFQ needed for inhibition is the portion fused upstream of *lacZ* (as opposed to the portion located downstream of *lacZ*).  
25 Presumably, only the region from the *Bam*HI site to nucleotide 3020 is needed in BGAFQ, since this is all that is contained in PBA.

A construct that contains the PBA *ced-3* portion but not any of the *lacZ* portion did not prevent cell death,  
30 suggesting that fusion to portions of *lacZ* is needed for expression or action of the inhibitory gene portion.

These observations indicate that the amino-terminal portion of the Ced-3 protein, possibly in conjunction with a portion of *E. coli*  $\beta$ -galactosidase, can act to  
35 prevent programmed cell deaths in *C. elegans*. One

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plausible mechanism is that this portion of the Ced-3 protein acts in a dominant negative or antimorphic fashion, to prevent the activity of the normal Ced-3 protein. (It is known that inactivation of the Ced-3 protein results in an absence of programmed cell deaths.) Such dominant negative activity could be a result of the partial Ced-3 protein binding to and, thereby, inactivating the normal Ced-3 protein; consistent with this model is the finding that the active form of the structurally similar ICE protein is dimeric. Alternatively, the partial Ced-3 protein may bind to a molecule with which the normal Ced-3 protein must interact to function and by preventing this interaction, inhibits Ced-3 activity.

Due to the structural similarity of ICE to the Ced-3 protein, fusion constructs encoding amino-terminal portions of ICE would also be expected to inhibit the activity of the *ced-3* gene. In particular, those portions of the ICE gene corresponding to the *ced-3* gene portions in BGAFQ and PBA, i.e., ICE codons 1 to 298 and codons 1 to 111, or active subportions of these, are expected to inhibit *ced-3*. A further extension of this reasoning suggests that corresponding gene portions of any structurally related *ced-3*/ICE family member would also have an inhibitory effect on *ced-3* activity.

Furthermore, the structural relatedness of the *ced-3* and ICE genes implies that the ICE enzyme could also be inhibited by fusion constructs containing amino-terminal portions of the ICE gene, as well as corresponding portions of other structurally related genes, such as *ced-3*.

Identification of portions of the *ced-3*, ICE, and related genes which inhibit the *ced-3* gene can be carried out by testing expression constructs containing these gene portions or their encoded products in

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bioassays for cell death activity. Identification of gene portions or encoded products which inhibit ICE can be carried out using previously described assays for ICE activity. For example: 1) wild-type worms can be  
5 injected with portions of the *ced-3* or other structurally related gene, such as ICE, to determine if they prevent programmed cell death; 2) portions of the ICE protein or other structurally similar protein, such as Ced-3, can be co-expressed with ICE and pro-IL-1 $\beta$  in  
10 nematodes or cultured mammalian cells to see if they inhibit ICE-catalyzed cleavage of the IL-1 $\beta$  precursor; and 3) peptides or nucleic acids containing portions of the amino acid or coding sequence of ICE or similar protein, such as Ced-3, can be tested using purified ICE  
15 and synthetic substrates.

Inhibitory portions of the *ced-3* gene, ICE, and structurally related genes, their encoded RNAs and proteins, and peptide and non-peptide mimetics of the proteins may be used to reduce cell deaths and/or  
20 inflammation, and are, thus, useful for the treatment of diseases involving these processes. The encoded proteins and peptide and non-peptide mimetics can be delivered by various known methods and routes of drug delivery. For example, they can be administered orally  
25 or by another parenteral route or by a non-parenteral route (e.g., by injection intramuscularly, intraperitoneally or intravenously or by topical administration). Alternatively, expression constructs containing the gene portions can be made using  
30 heterologous transcriptional and translational signals or signals native to the gene portions. The constructs can be delivered into cells by various methods of gene therapy, such as retroviral infection.

Interestingly, those ICE gene portions  
35 corresponding to the *ced-3* portions of BGAFO and PBA

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encode approximately the protein fragments which result from cleavage at each of the two autocleavage sites (amino acids 103 and 297). This observation suggests that autoproteolytic conversion of the proenzyme to  
5 active ICE involves cleaving off the inhibitory amino-terminal portions of the protein. Active ICE is a heterodimer composed of subunits of about 20 and 10 kilodaltons (Thornberry et al., *Nature* 356:768-774 (1992)). These subunits have been shown to be derived  
10 from the ICE proenzyme and correspond to amino acids 120 to 297 (p20) and 317 to 404 (p10). Kinetic studies suggest that association of the two subunits is required for activity of the enzyme. It is possible that the amino-terminal region of the protein interferes with  
15 this association.

This implies that mutant proteins in which the inhibitory amino-terminal regions are deleted may be constitutively activated. Thus, carboxyl-terminal portions of the Ced-3, ICE, and related proteins, and  
20 constructs and RNAs expressing these portions, are potentially useful for increasing cell deaths and/or IL-1 $\beta$  production. Constructs which may be used include those which express the carboxyl region of ICE, which encodes the two subunits of the active enzyme, as well  
25 as those which express each of these subunits separately. In addition, it is possible that the amino region of ICE, which is not needed for ICE enzymatic activity *in vitro*, is important for ICE activity or the regulation of ICE activity *in vivo*. Consistent with  
30 this idea is the finding that two of the *ced-3* mutations map in this region. For this reason, a construct which expresses the amino region of Ced-3, ICE or a Ced-3/ICE gene family member may also be used. Furthermore, the NEDD-2 protein, which is similar to a carboxyl-terminal  
35 portion of the Ced-3 portion, may also exhibit

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constitutive activity in causing cell deaths. Thus, all or active portions of NEDD-2, and DNA and RNA encoding NEDD-2 proteins, would be expected to produce cell death activity when expressed. Drugs comprising activated  
5 molecules derived from the carboxyl-terminal regions of Ced-3, ICE and other proteins of the Ced-3/ICE family and from the NEDD-2 protein, DNAs and RNAs encoding these proteins and protein fragments, as well as peptide and non-peptide mimetics, are potentially useful for  
10 controlling or reducing the size of undesirable cell populations, such as cancerous cells, infected cells, cells producing autoreactive antibodies and hair follicle cells. Such drugs may also be useful for incapacitating or killing undesired organisms, such as  
15 parasites, pests, and genetically engineered organisms. For example, a number of nematodes are human, animal and plant parasites.

#### ICE Inhibitors As Inhibitors of Cell Death

The conservation of the active site of ICE (active  
20 cysteine and surrounding amino acids) in the Ced-3 protein implies that Ced-3 is a cysteine protease which interacts with its substrate by a similar mechanism. Hence, it is likely that inhibitors of ICE which interfere with this mechanism, or chemical analogs of  
25 these inhibitors, will also inhibit Ced-3 function.

Peptide aldehydes containing the ICE recognition site:

30 P4--P3--P2--P1  
Tyr-Val-Ala-Asp

or a substituted site in which P2 is Ala, His, Gln, Lys, Phe, Cha, or Asp, have been shown to be effective, specific, and reversible inhibitors of the protease activity of ICE (Thornberry et al., Nature 356:768-774

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(1992)). These molecules are thought to act as transition analogs, which compete for ICE binding to its substrate, pro-IL-1 $\beta$ . Three such inhibitors have been described: Inhibitor B (Ac-Tyr-Val-Ala-Asp-CHO);

- 5 Inhibitor C (Ac-Tyr-D-Ala-Ala-Asp-CHO); and Inhibitor D (Ac-Tyr-Val-Lys-Asp-CHO). Of these, Inhibitor B is the most potent, with a  $K_i$ =0.76 nM compared to  $K_i$ =3 nM for D and  $K_i$ =1.5  $\mu$ M for C.

In addition, the *crmA* gene of cowpox virus has been  
10 found to encode a serpin which specifically inhibits ICE (Ray et al., *Cell* 69:597-604 (1992)). The serpin acts by preventing the proteolytic activation of ICE. This inhibitor of ICE is also expected to inhibit structurally similar proteins, such as Ced-3. The *crmA*  
15 gene and methods for obtaining purified CrmA protein have been described (Pickup et al., *Proc. Natl. Acad. Sci. USA* 83:7698-7702 (1986); Ray et al., 1992 *supra*). This invention includes the use of inhibitors of ICE, such as peptide aldehydes, and particularly inhibitor B,  
20 and the CrmA protein, as drugs for decreasing the activity of cell death genes and, thus, for treatment of diseases characterized by cell deaths.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation,  
25 many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. For example, functional equivalents of DNAs and RNAs may be nucleic acid sequences which, through the degeneracy of  
30 the genetic code, encode the same proteins as those specifically claimed. Functional equivalents of proteins may be substituted or modified amino acid sequences, wherein the substitution or modification does not change the activity or function of the protein. A

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"silent" amino acid substitution, such that a chemically similar amino acid (e.g., an acidic amino acid with another acidic amino acid) is substituted, is an example of how a functional equivalent of a protein can be produced. Functional equivalents of nucleic acids or proteins may also be produced by deletion of nonessential sequences.

The following examples illustrate the invention and are not intended to be limiting in any way.

10

EXAMPLE 1CLONING, SEQUENCING, AND CHARACTERIZATION OF  
THE CED-3 GENE

## MATERIALS AND METHODS

General Methods and Strains

15 The techniques used for the culturing of *C. elegans* were as described by Brenner (*Genetics* 77:71-94 (1974)). All strains were grown at 20°C. The wild-type parent strains were *C. elegans* variety Bristol strain N2, Bergerac strain EM1002 (Emmons et al., *Cell* 32:55-65  
20 (1983)), *C. briggsae* and *C. vulgaris* (obtained from V. Ambros). The genetic markers used are described below. These markers have been described by Brenner (1974 *supra*), and Hodgkin et al. (In: *The Nematode Caenorhabditis elegans*, Wood and the Community of *C. elegans* Researchers (eds.), Cold Spring Harbor  
25 Laboratory, 1988, pp 491-584). Genetic nomenclature follows the standard system (Horvitz et al., *Mol. Gen. Genet.* 175:129-133 (1979)):

LG I: *ced-1(e1375); unc-54(r323)*

30 LG VI: *unc-31(e928), unc-30(e191), ced-3(n717, n718, n1040, n1129, n1163, n1164, n1165, n1286,*

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n1949, n2426, n2430, n2433), *unc-26(e205)*,  
*dpy-4(e1166)*

LG V: *egl-1(n986)*; *unc-76(e911)*

LG X: *dpy-3(e27)*

#### 5 Isolation of Additional Alleles of *ced-3*

A non-complementation screen was designed to isolate new alleles of *ced-3*. Because animals heterozygous for *ced-3(n717)* in trans to a deficiency are viable (Ellis and Horvitz, Cell 44:817-829 (1986)),  
10 animals carrying a complete loss-of-function *ced-3* allele generated by mutagenesis were expected to be viable in trans to *ced-3(n717)*, even if the new allele was inviable in homozygotes. Fourteen EMS mutagenized *egl-1* males were mated with *ced-3(n717) unc-26(e205)*;  
15 *egl-1(n487)*; *dpy-3(e27)* hermaphrodites. *egl-1* was used as a marker in this screen. Dominant mutations in *egl-1* cause the two hermaphrodite specific neurons, the HSNs, to undergo programmed cell death (Trent et al., Genetics 104:619-647 (1983)). The HSNs are required for normal  
20 egg-laying, and *egl-1(n986)* hermaphrodites, which lack HSNs, are egg-laying defective (Trent et al., 1983 *supra*). The mutant phenotype of *egl-1* is suppressed in a *ced-3*; *egl-1* strain because mutations in *ced-3* block programmed cell deaths. *egl-1* males were mutagenized  
25 with EMS and crossed with *ced-3(n717)*, *unc-26(e205)*; *egl-1(n487)*; *dpy-3(e27)*. Most cross progeny were egg-laying defective because they were heterozygous for *ced-3* and homozygous for *egl-1*. Rare egg-laying competent animals were picked as candidates for carrying new  
30 alleles of *ced-3*. Four such animals were isolated from about 10,000 F1 cross progeny of EMS-mutagenized animals. These new mutations were made homozygous to confirm that they carried recessive mutations of *ced-3*.



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Molecular Biology

Standard techniques of molecular biology were used (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1983).

5 Two cosmid libraries were used extensively in this work: a *Sau3AI* partial digest genomic library of 7000 clones in the vector pHC79 and a *Sau3AI* partial digest genomic library of 6000 clones in the vector pJB8 (Ish-Horowicz and Burke, *Nucleic Acids Res.* 9:2989 (1981)).

10 The "right" end of MMM-C1 was cloned by cutting it with *HindIII* and self-ligating. The "left" end of MMM-C1 was cloned by cutting it with *BglII* or *SalI* and self-ligating.

The "right" end of Jc8 was made by digesting Jc8  
15 with *EcoRI* and self-ligating. The "left" end of Jc8 was made by digesting Jc8 by *SalI* and self-ligating.

*C. elegans* RNA was extracted using guanidine isothiocyanate (Kim and Horvitz, *Genes & Dev.* 4:357-371 (1990)). Poly(A)<sup>+</sup> RNA was selected from total RNA by a  
20 poly(dT) column (Maniatis et al., 1983 *supra*). To prepare stage-synchronized animals, worms were synchronized at different developmental stages (Meyer and Casson, *Genetics* 106:29-44 (1986)).

For DNA sequencing, serial deletions were made  
25 according to a procedure developed by Henikoff (*Gene* 28:351-359 (1984)). DNA sequences were determined using Sequenase and protocols obtained from US Biochemicals with minor modifications.

The *Tcl* DNA probe for Southern blots was pCe2001,  
30 which contains a Bergerac *Tcl* element (Emmons et al., *Cell* 32:55-65 (1983)). Enzymes were purchased from New England Biolabs, and radioactive nucleotides were from Amersham.

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Primer extension procedures followed the procedure by Robert E. Kingston (In: *Current Protocols in Molecular Biology*, Ausubel et al. (eds.), Greene Publishing Associates and Wiley-Interscience, New York, p. 4.8.1) with minor modifications.

Polymerase chain reaction (PCR) was carried out using standard protocols supplied by the GeneAmp Kit (Perkin Elmer). The primers used for primer extension and PCR are as follows:

- 10 Pex2: 5' TCATCGACTTTTAGATGACTAGAGAACATC 3'  
(SEQ ID NO:7);
- Pex1: 5' GTTGCACTGCTTTCACGATCTCCCGTCTCT 3'  
(SEQ ID NO:8);
- SL1: 5' GTTTAATTACCCAAGTTTGAG 3' (SEQ ID NO:9);
- 15 SL2: 5' GGTTTAAACCAGTTACTCAAG 3' (SEQ ID NO:10);
- Log5: 5' CCGGTGACATTGGACACTC 3' (SEQ ID NO:11); and
- Oligo10: 5' ACTATTCAACACTTG 3' (SEQ ID NO:12).

#### Germline Transformation

The procedure for microinjection basically follows that of A. Fire (*EMBO J.* 5:2673-2680 (1986)) with modifications: Cosmid DNA was twice purified by CsCl-gradient. Miniprep DNA was used when deleted cosmids were injected. To prepare miniprep DNA, DNA from 1.5 ml overnight bacterial culture in superbrot (12 g Bacto-tryptone, 24 g yeast extract, 8 ml 50% glycerol, 900 ml H<sub>2</sub>O, autoclaved; after autoclaving, 100 ml 0.17 M KH<sub>2</sub>PO<sub>4</sub> and 0.72 M KH<sub>2</sub>PO<sub>4</sub> were added) was extracted by alkaline lysis method as described in Maniatis et al. (1983 *supra*). DNA was treated with RNase A (37°, 30 minutes) and then with protease K (55°, 30 minutes), extracted with phenol and then chloroform, precipitated twice (first in 0.3 M sodium acetate and second in 0.1 M

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potassium acetate, pH 7.2), and resuspended in 5  $\mu$ l injection buffer as described by A. Fire (1986 *supra*). The DNA concentration for injection is in the range of .00 ug to 1 mg per ml.

All transformation experiments used *ced-1(e1735); unc-31(e928) ced-3(n717)* strain. *unc-31* was used as a marker for co-transformation (Kim and Horvitz, 1990 *supra*). *ced-1* was present to facilitate scoring of the Ced-3 phenotype. The mutations in *ced-1* block the engulfment process of cell death, which makes the corpses of the dead cells persist much longer than in wild-type animals (Hedgecock et al., *Science* 220:1277-1280 (1983)). The Ced-3 phenotype was scored as the number of dead cells present in the head of young L1 animals. The cosmid C10D8 or the plasmid subclones of C10D8 were mixed with C14G10 (*unc-31(+)*-containing) at a ratio of 2:1 or 3:1 to increase the chances that a *Unc-31(+)* transformant would contain the cosmid or plasmid being tested as well. Usually, 20-30 animals were injected in one experiment. Non-*Unc* F1 progeny of the injected animal were isolated three to four days later. About 1/2 to 1/3 of the non-*Unc* progeny transmitted the non-*Unc* phenotype to F2 progeny and established a transformant line. The young L1 progeny of such non-*Unc* transformant were checked for the number of dead cells present in the head using Nomarski optics.

## RESULTS

### Isolation of Additional *ced-3* Alleles

All of the *ced-3* alleles that existed previously were isolated in screens designed to detect viable mutants displaying the Ced phenotype (Ellis and Horvitz, *Cell* 44:817-829 (1986)). Such screens may have systematically missed any class of *ced-3* mutations that

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is inviable as homozygotes. For this reason, a scheme was designed that could isolate recessive lethal alleles of *ced-3*. Four new alleles of *ced-3* (*n1163*, *n1164*, *n1165*, *n1286*) were isolated in this way. Since new  
5 alleles were isolated at a frequency of about 1 in 2500, close to the frequency expected for the generation of null mutations by EMS in an average *C. elegans* gene (Brenner, *Genetics* 77:71-94 (1974); Greenwald and Horvitz, *Genetics* 96:147-160 (1980)), and all four  
10 alleles are homozygous viable, it was concluded that the null allele of *ced-3* is viable.

#### Mapping RFLPs near *ced-3*

*Tc1* is a *C. elegans* transposable element that is thought to be immobile in the common laboratory Bristol  
15 strain and in the Bergerac strain (Emmons et al., *Cell* 32:55-65 (1983)). In the Bristol strain, there are 30 copies of *Tc1*, while in the Bergerac strain, there are more than 400 copies of *Tc1* (Emmons et al., 1983 *supra*; Finney, Ph.D. thesis, Massachusetts Institute of  
20 Technology, Cambridge, Massachusetts, 1987). Because the size of the *C. elegans* genome is small (haploid genome size  $8 \times 10^7$  bp) (Sulston and Brenner, *Genetics* 77:95-104 (1976)), a polymorphism due to *Tc1* between the Bristol and Bergerac strains would be expected to occur  
25 about once every 200 kb. Restriction fragment length polymorphisms (RFLPs) can be used as genetic markers and mapped in a manner identical to conventional mutant phenotypes. A general scheme has been designed to map  
30 *Tc1* elements that are dimorphic between the Bristol and Bergerac strains near any gene of interest (Ruvkun et al., *Genetics* 121:501-516 (1989)). Once tight linkage of a particular *Tc1* to a gene of interest has been

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established, that Tc1 can be cloned and used to initiate chromosome walking.

A 5.1 kb Bristol-specific Tc1 *EcoRI* fragment was tentatively identified as containing the Tc1 closest to  
5 *ced-3*. This Tc1 fragment was cloned using cosmids from a set of Tc1-containing *C. elegans* Bristol genomic DNA fragments. DNA was prepared from 46 such Tc1-containing cosmids and screened using Southern blots to identify the cosmids that contain a 5.1 kb *EcoRI* Tc1-containing  
10 fragment. Two such cosmids were identified: MMM-C1 and MMM-C9. The 5.1 kb *EcoRI* fragment was subcloned from MMM-C1 into pUC13 (Promega). Since both ends of Tc1 contain an *EcoRV* site (Rosenzweig et al., *Nucleic Acids Res.* 11:4201-4209 (1983)), *EcoRV* was used to remove Tc1  
15 from the 5.1 kb *EcoRI* fragment, generating a plasmid that contains only the unique flanking region of this Tc1-containing fragment. This plasmid was then used to map the specific Tc1 without the interference of other Tc1 elements.

20 *unc-30(e191) ced-3(n717) dpy-4(e1166)/+++* males were crossed with Bergerac (EM1002) hermaphrodites, and Unc non-Dpy or Dpy non-Unc recombinants were picked from among the F2 progeny. The recombinants were allowed to self-fertilize, and strains that were homozygous for  
25 either *unc-30(e191) dpy-4(Bergerac)* or *unc-30(Bergerac) dpy-4(e1166)* were isolated. After identifying the *ced* genotypes of these recombinant strains, DNA was prepared from these strains. A Southern blot of DNA from these recombinants was probed with the flanking sequence of  
30 the 5.1 kb *EcoRI* Tc1 fragment. This probe detects a 5.1 kb fragment in Bristol N2 and a 3.4 kb fragment in Bergerac. Five out of five *unc-30 ced-3 dpy(+Berg)* recombinants, and one of one *unc-30(+Berg) ced-3 dpy-4* recombinants showed the Bristol pattern. Nine of ten  
35 *unc-30(+Berg) dpy-4* recombinants showed the Bergerac

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pattern. Only one recombinant of *unc-30(+Berg)* *dpy-4* resulted from a cross-over between *ced-3* and the 5.1 kb *Tcl* element. The genetic distance between *ced-3* and *dpy-4* is 2 map units ( $\mu$ ). Thus, this *Tcl* element is  
5 located 0.1  $\mu$  on the right side of *ced-3*.

Cosmids MMM-C1 and MMM-C9 were used to test whether any previously mapped genomic DNA cosmids overlapped with these two cosmids. A contig of overlapping cosmids was identified that extended the cloned region near *ced-*  
10 3 in one direction.

To orient MMM-C1 with respect to this contig, both ends of MMM-C1 were subcloned and these subclones were used to probe the nearest neighboring cosmid C48D1. The "right" end of MMM-C1 does not hybridize to C48D1, while  
15 the "left" end does. Therefore, the "right" end of MMM-C1 extends further away from the contig. To extend this contig, the "right" end of MMM-C1 was used to probe the filters of two cosmid libraries (Coulson et al., *Proc. Natl. Acad. Sci. USA* 83:7821-7825 (1986)). One clone,  
20 Jc8, was found to extend MMM-C1 in the opposite direction of the contig.

RFLPs between the Bergerac and Bristol strains were used to orient the contig with respect to the genetic map. Bristol (N2) and Bergerac (EM1002) DNA was  
25 digested with various restriction enzymes and probed with different cosmids to look for RFLPs. Once such an RFLP was found, DNA from recombinants of the Bristol and Bergerac strains between *ced-3* and *unc-26*, and between *unc-30* and *ced-3* was used to determine the position of  
30 the RFLP with respect to *ced-3*.

The "right" end of Jc8, which represents one end of the contig, detects an RFLP (*nP33*) when N2 and EM1002 DNA was digested with *HindIII*. A Southern blot of DNA from recombinants between three *ced-3(+Berg)* *unc-26* was  
35 probed with the "right" end of Jc8. Three of three

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+Berg *unc-26* recombinants showed the Bristol pattern, while two of two *ced-3 unc-26(+Berg)* recombinants showed the Bergerac pattern. Thus, *nP33* mapped very close or to the right side of *unc-26*.

5       The "left" end of Jc8 also detects a *HindIII* RFLP (*nP34*). The same Southern blot was reprobed with the Jc8 "left" end. Two of the two *ced-3 unc-26(+Berg)* recombinants and two of the three *ced-3(+Berg) unc-26* recombinants showed the Bergerac pattern. One of the  
10 three *ced-3(+Berg) unc-26* recombinants showed the Bristol pattern. The genetic distance between *ced-3* and *unc-26* is 0.2 mu. Thus, *nP34* was mapped between *ced-3* and *unc-26*, about 0.1 mu on the right side of *ced-3*.

      The flanking sequence of the 5.1 kb *EcoRI* Tc1  
15 fragment (named *nP35*) was used to probe the same set of recombinants. Two of three *ced-3(+Berg) unc-26* recombinants and two of two *ced-3 unc-26(+Berg)* recombinants showed the Bristol pattern. Thus, *nP35* was also found to be located between *ced-3* and *unc-26*, about  
20 0.1 mu on the right side of *ced-3*.

      A similar analysis using cosmid T10H5 which contains the *HindIII* RFLP (*nP36*), and cosmid B0564, which contains a *HindIII* RFLP (*nP37*), showed that *nP36* and *nP37* mapped very close or to the right of *unc-30*.

25       These experiments localized the *ced-3* gene to an interval of three cosmids. The positions of the RFLPs, and of *ced-3*, *unc-30* and *unc-26* on chromosome IV, and their relationships to the cosmids are shown in Figure 1. It has been demonstrated by microinjection that  
30 cosmids C37G8 and C33F2 carry the *unc-30* gene (John Sulston, personal communication). Thus, the region containing the *ced-3* gene was limited to an interval of two cosmids. These results are summarized in Figure 1.

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Complementation of *ced-3* by Germline Transformation

Cosmids that were candidates for containing the *ced-3* gene were microinjected into a *ced-3* mutant to see if they rescue the mutant phenotype. The procedure for microinjection was that of A. Fire (*EMBO J.* 5:2673-2680 (1986)) with modifications. *unc-31*, a mutant defective in locomotion, was used as a marker for cotransformation (Kim and Horvitz, *Genes & Dev.* 4:357-371 (1990)), because the phenotype of *ced-3* can be examined only by using Nomarski optics. Cosmid C14G10 (containing *unc-31(+)*) and a candidate cosmid were coinjected into *ced-1(e1375); unc-31(e928) ced-3(n717)* hermaphrodites, and F1 non-Unc transformants were isolated to see if the non-Unc phenotype could be transmitted and established as a line of transformants. Young L1 progeny of such transformants were examined for the presence of cell deaths using Nomarski optics to see whether the Ced-3 phenotype was suppressed. Cosmid C14G10 containing *unc-31* alone does not rescue *ced-3* activity when injected into a *ced-3* mutant. Table 4 summarizes the results of these transformation experiments.

As shown in Table 3, of the three cosmids injected (C43C9, W07H6 and C48D1), only C48D1 rescued the Ced-3 phenotype (2/2 non-Unc transformants rescued the Ced-3 phenotype). One of the transformants, *nEX2*, appears to be rescued by an extra-chromosomal array of injected cosmids (Way and Chalfie, *Cell* 54:5-16 (1988)), which is maintained as an unstable duplication, since only 50% of the progeny of a non-Unc Ced(+) animal are non-Unc Ced(+). Since the non-Unc Ced(+) phenotype of the other transformant (*nIS1*) is transmitted to all of its progeny, it is presumably an integrated transformant. L1 *ced-1* animals contain an average of 23 cell corpses in the head. L1 *ced-1; ced-3* animals contain an average of 0.3 cell corpses in the head. *ced-1; unc-31 ced-3; nIS1* and *ced-1; unc-31 ced-3; nEX2* animals contain an



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average of 16.4 and 14.5 cell corpses in the head, respectively. From these results, it was concluded that C48D1 contains the *ced-3* gene.

In order to locate *ced-3* more precisely within the cosmid C48D1, this cosmid was subcloned and the subclones were tested for the ability to rescue *ced-3* mutants. C48D1 DNA was digested with restriction enzymes that cut rarely within the cosmid and the remaining cosmid was self-ligated to generate a subclone. Such subclones were then injected into a *ced-3* mutant to look for completion. When C48D1 was digested with *Bam*HI and self-ligated, the remaining 14 kb subclone (named C48D1-28) was found to rescue the *Ced-3* phenotype when injected into a *ced-3* mutant (Figure 2 and Table 4). C48D1-28 was then partially digested with *Bgl*III and self-ligated. Clones of various lengths were isolated and tested for their ability to rescue *ced-3*.

One clone, C48D1-43, which did not contain a 1.7 kb *Bgl*III fragment of C48D1-28, was able to rescue *ced-3* (Figure 2 and Table 4). C48D1-43 was further subcloned by digesting with *Bam*HI and *Apa*I to isolate a 10 kb *Bam*HI-*Apa*I fragment. This fragment was subcloned into pBSKII+ to generate pJ40. pJ40 can restore *Ced-3*+ phenotype when microinjected into a *ced-3* mutant. pJ40 was subcloned by deleting a 2 kb *Bgl*III-*Apa*I fragment to generate pJ107. pJ107 was also able to rescue the *Ced-3* phenotype when microinjected into a *ced-3* mutant. Deletion of 0.5 kb on the left side of pJ107 could be made by *Exo*III digestion (as in pJ107del28 and pJ107del34) without affecting *Ced-3* activity; in fact, one transgenic line, *nEX17*, restores full *Ced-3* activity. However, the *ced-3* rescuing ability was significantly reduced when 1 kb was deleted on the left side of pJ107 (as in pJ107del12 and pJ107del27), and the

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ability was completely eliminated when a 1.8 kb *SaII*-*BglIII* fragment was deleted on the right side of pJ107 (as in pJ55 and pJ56), suggesting that this *SaII* site is likely to be in the *ced-3* coding region. From these  
5 experiments, *ced-3* was localized to a DNA fragment of 7.5 kb. These results are summarized in Figure 2 and Table 4.

#### *ced-3* Transcript

pJ107 was used to probe a Northern blot of N2 RNA  
10 and detected a band of 2.8 kb. Although this transcript is present in 12 *ced-3* mutant animals, subsequent analysis showed that all 12 *ced-3* mutant alleles contain mutations in the genomic DNA that codes for this mRNA (see below), thus establishing this RNA as a *ced-3*  
15 transcript.

The developmental expression pattern of *ced-3* was determined by hybridizing a Northern blot of RNA from animals of different stages (eggs, L1 through L4 larvae and young adult) with the *ced-3* cDNA subclone pJ118.  
20 Such analysis revealed that the *ced-3* transcript is most abundant during embryonic development, which is the period when most programmed cell deaths occur, but it was also detected during the L1 through L4 larval stages and is present in relatively high levels in young  
25 adults. This result suggests that *ced-3* is not only expressed in cells undergoing programmed cell death.

Since *ced-3* and *ced-4* are both required for programmed cell death in *C. elegans*, one of the genes might act as a regulator of transcription of the other  
30 gene. To examine if *ced-4* regulates the transcription of *ced-3*, RNA was prepared from eggs of *ced-4* mutants (*n1162*, *n1416*, *n1894*, and *n1920*), and a Northern blot was probed with the *ced-3* cDNA subclone pJ118. The presence of RNA in each lane was confirmed with an actin

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I probe. Such an experiment showed that the level of *ced-3* transcript is normal in *ced-4* mutants. This indicates that *ced-4* is unlikely to be a transcriptional regulator of *ced-3*.

#### 5 Isolation of a *ced-3* cDNA

To isolate cDNA of *ced-3*, pJ40 was used as a probe to screen a cDNA library of N2 (Kim and Horvitz, *Genes & Dev.* 4:357-371 (1990)). Seven cDNA clones were isolated. These cDNAs can be divided into two groups:  
10 one is 3.5 kb and the other 2.5 kb. One cDNA from each group was subcloned and analyzed further. pJ85 contains the 3.5 kb cDNA. Experiments showed that pJ85 contains a *ced-3* cDNA fused to an unrelated cDNA; on Northern blots of N2 RNA, the pJ85 insert hybridizes to two RNA  
15 transcripts, and on Southern blots of N2 DNA, pJ85 hybridizes to one more band than pJ40 (*ced-3* genomic DNA) does. pJ87 contains the 2.5 kb cDNA. On Northern blots, pJ87 hybridizes to a 2.8 kb RNA and on Southern blots, it hybridizes only to bands to which pJ40  
20 hybridizes. Thus, pJ87 contains only *ced-3* cDNA.

To show that pJ87 does contain the *ced-3* cDNA, a frameshift mutation was made in the *Sa*I site of pJ40 corresponding to the *Sa*I site in the pJ87 cDNA. Constructs containing the frameshift mutation failed to  
25 rescue the *Ced-3* phenotype when microinjected into *ced-3* mutant animals, suggesting that *ced-3* activity has been eliminated.

#### *ced-3* Sequence

The DNA sequence of pJ87 was determined (Figure 3).  
30 pJ87 contains an insert of 2.5 kb which has an open reading frame of 503 amino acids (Figure 3; SEQ ID NO:2). The 5' end of the cDNA contains 25 bp of poly-A/T sequence, which is probably an artifact of cloning

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and is not present in the genomic sequence. The cDNA ends with a poly-A sequence, suggesting that it contains the complete 3' end of the transcript. 1 kb of pJ87 insert is untranslated 3' region and not all of it is  
5 essential for *ced-3* expression, since genomic constructs with deletions of 380 bp of the 3' end can still rescue *ced-3* mutants (pJ107 and its derivatives, see Figure 2).

To confirm the DNA sequence obtained from the *ced-3* cDNA and to study the structure of the *ced-3* gene, the  
10 genomic sequence of the *ced-3* gene in the plasmid pJ107 was determined (Figure 3; SEQ ID NO:1). Comparison of the *ced-3* genomic and cDNA sequences revealed that the *ced-3* gene has seven introns that range in size from 54 bp to 1195 bp (Figure 4A). The four largest introns, as  
15 well as sequences 5' of the start codon, were found to contain repetitive elements (Figure 3). Five types of repetitive elements were found, some of which have been previously characterized in non-coding regions of other *C. elegans* genes, such as *fem-1* (Spence et al., *Cell*  
20 60:981-990 (1990)), *lin-12* (J. Yochem, personal communication), and *myoD* (Krause et al., *Cell* 63:907-919 (1990)). Of these, repeat 1 was also found in *fem-1* and *myoD*, repeat 3 in *lin-12* and *fem-1*, repeat 4 in *lin-12*, and repeats 2 and 5 were novel repetitive elements.

25 A combination of primer extension and PCR amplification was used to determine the location and nature of the 5' end of the *ced-3* transcript. Two primers (Pex1 and Pex2) were used for the primer extension reaction. The Pex1 reaction yielded two major  
30 bands, whereas the Pex2 reaction gave one band. The Pex2 band corresponded in size to the smaller band from the Pex1 reaction, and agreed in length with a possible transcript that is trans-spliced to a *C. elegans* splice leader (Bektesh, *Genes & Devel.* 2:1277-1283 (1988)) at a  
35 consensus splice acceptor at position 2166 of the

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genomic sequence (Figure 3). The nature of the larger Pex1 band is unclear.

To confirm the existence of this trans-spliced message in wild-type worms, total *C. elegans* RNA was PCR  
5 amplified using the SL1-Log5 and SL2-Log5 primer pairs, followed by a reamplification using the SL1-Oligo10 and SL2-Oligo10 primer pairs. The SL1 reaction yielded a fragment of the predicted length. The identity of this  
10 fragment was confirmed by sequencing. Thus, at least some, if not most, of the *ced-3* transcript is trans-spliced to SL1. Based on this result, the start codon of the *ced-3* message was assigned to the methionine encoded at position 2232 of the genomic sequence (Figure 3).

15 The DNA sequences of 12 EMS-induced *ced-3* alleles were also determined (Figure 3 and Table 1). Nine of the 12 are missense mutations. Two of the 12 are nonsense mutations, which might prematurely terminate the translation of *ced-3*. These nonsense *ced-3* mutants  
20 confirmed that the *ced-3* gene is not essential for viability. One of the 12 mutations is an alteration of a conserved splicing acceptor G, and another has a change of a 70% conserved C at the splice site, which could also generate a stop codon even if the splicing is  
25 correct. Interestingly, these EMS-induced mutations are in either the N-terminal quarter or C-terminal half of the protein. In fact, 9 of the 12 mutations occur within the region of *ced-3* that encodes the last 100 amino acids of the protein. Mutations are notably  
30 absent from the middle part of the *ced-3* gene (Figure 4A).

#### Ced-3 Protein Contains A Region Rich in Serines

The Ced-3 protein is very hydrophilic and no  
35 significantly hydrophobic region can be found that might

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be a trans-membrane domain (Figure 5). The Ced-3 protein is rich in serine. From amino acid 78 to amino acid 205 of the Ced-3 protein, 34 out of 127 amino acids are serine. Serine is often the target of

5 serine/threonine protein kinases (Edelman, *Ann. Rev. Biochem.* 56:567-613 (1987)). For example, protein kinase C can phosphorylate serines when they are flanked on their amino and carboxyl sides by basic residues (Edelman, 1987 *supra*). Four of the serines in the Ced-3

10 protein are flanked by arginines (Figures 6A-B). The same serine residues might also be the target of related Ser/Thr kinases.

To identify the functionally important regions of the Ced-3 protein, genomic DNAs containing the *ced-3*

15 genes from two related nematode species, *C. briggsae* (SEQ ID NO:5) and *C. vulgaris* (SEQ ID NO:6) were cloned and sequenced. Sequence comparison of the three *ced-3* gene products showed that the non-serine-rich region of the proteins is highly conserved (Figure 9). In *C.*

20 *briggsae* and *C. vulgaris*, many amino acids in the serine-rich region are dissimilar compared to the *C. elegans* Ced-3 protein. It seems that what is important in the serine-rich region is the overall serine-rich feature rather than the exact amino acid sequence.

25 This hypothesis is also supported by analysis of *ced-3* mutations in *C. elegans*: none of the 12 EMS-induced mutations is in the serine-rich region, suggesting that mutations in this region might not affect the function of the Ced-3 protein and thus, could

30 not be isolated in the screen for *ced-3* mutants.

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EXAMPLE 2A COMMON MECHANISM OF CELL DEATH  
IN VERTEBRATES AND INVERTEBRATES

Results from previous studies reported in the  
5 scientific literature suggest that cell deaths in a  
variety of organisms, including vertebrates as well as  
invertebrates, share a common mechanism which involves  
the activation of genes. These studies are consistent  
with the hypothesis that genes similar to the *C. elegans*  
10 *ced-3* and *ced-4* genes may be involved in the cell deaths  
that occur in vertebrates, although certain observations  
have led some to distinguish vertebrate cell deaths from  
the programmed cell deaths observed in such  
invertebrates as nematodes and insects. Some vertebrate  
15 cell deaths share certain characteristics with the  
programmed cell deaths in *C. elegans* that are controlled  
by *ced-3* and *ced-4*. For example, up to 14% of the  
neurons in the chick dorsal root ganglia die immediately  
after their births, before any signs of differentiation  
20 (Carr and Simpson, *Dev. Brain Res.* 2:57-162 (1982)).  
Genes like *ced-3* and *ced-4* could well function in this  
class of vertebrate cell death.

Genetic mosaic analysis has suggested that *ced-3*  
and *ced-4* genes are expressed by cells that undergo  
25 programmed cell death, so that these genes may not act  
through cell-cell interactions (Yuan and Horvitz, *Dev.*  
*Biol.* 138:33-41 (1990)). Many cell deaths in  
vertebrates seem different in that they appear to be  
controlled by interactions with target tissues. For  
30 example, it is thought that a deprivation of target-  
derived growth factors is responsible for vertebrate  
neuronal cell deaths (Hamburger and Oppenheim, *Neurosci.*  
*Comment.* 1:39-55 (1982)); Thoenen et al., in: *Selective*  
*Neuronal Death*, Wiley, New York, 1987, Vol. 126, pp. 82-

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85). However, even this class of cell death could involve genes like *ced-3* and *ced-4*, since pathways of cell death involving similar genes and mechanisms might be triggered in a variety of ways. Supporting this idea are several *in vitro* and *in vivo* studies which show that the deaths of vertebrate as well as invertebrate cells can be prevented by inhibitors of RNA and protein synthesis, suggesting that activation of genes are required for these cell deaths (Martin et al., *J. Cell Biol.* 106:829-844 (1988); Cohen and Duke, *J. Immunol.* 132:38-42 (1984); Oppenheim and Prevette, *Neurosci. Abstr.* 14:368 (1988); Stanisic et al., *Invest. Urol.* 16:19-22 (1978); Oppenheim et al., *Dev. Biol.* 138:104-113 (1990); Fahrbach and Truman, in: *Selective Neuronal Death, Ciba Foundation Symposium*, 1987, No. 126, pp. 65-81). It is possible that the genes induced in these dying vertebrate and invertebrate cells are cell death genes which are structurally related to the *C. elegans* *ced-3* or *ced-4* genes.

Also supporting the hypothesis that cell death in *C. elegans* is mechanistically similar to cell death in vertebrates is the observation that the protein product of the *C. elegans* gene *ced-9* is similar in sequence to the human protein Bcl-2. *ced-9* has been shown to prevent cells from undergoing programmed cell death during nematode development by antagonizing the activities of *ced-3* and *ced-4* (Hengartner, et al., *Nature* 356:494-499 (1992)). The *bcl-2* gene has also been implicated in protecting cells against cell death. It seems likely that the genes and proteins with which *ced-9* and *bcl-2* interact are similar as well.



**Table 1**  
**Sites of Mutations in the ced-3 Gene**

<u>Allele</u>	<u>Mutation</u>	<u>Nucleotide</u>	<u>Codon</u>	<u>Consequence</u>
n1040	C to T	2310	27	L to F
n718	G to A	2487	65	G to R
n2433	G to A	5757	360	G to S
n1164	C to T	5940	403	Q to termination
n717	G to A	6297	-	Splice acceptor loss
n1949	C to T	6322	412	Q to termination
n1286	G to A	6342	428	W to termination
n1129	C to T	6434	449	A to V
n1165	C to T	6434	449	A to V
n2430	C to T	6485	466	A to V
n2426	G to A	6535	483	E to K
n1163	C to T	7020	486	S to F

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Nucleotide and codon positions correspond to the numbering in Figure 3.

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Table 2  
ced-3-lacZ Fusions Which  
Prevent Programmed Cell Death

<u>Strain Name</u>	<u>Construct</u>	<u>Average # Extra Cells</u>	<u>Number of Animals</u>
N2 (wild-type)	-	0.1	40
nEx 121	PBA	2.0	23
nEx 70	PBA	2.4	31
nEx 67	BGAFQ	2.1	18
nEx 66	BGAFQ	2.1	25

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**Table 3**  
**Summary of Transformation Experiments**  
**Using Cosmids in the *ced-3* Region**

<u>Cosmid injected</u>	<u>No. of non-Unc transformants</u>	<u>Ced-3 phenotype</u>	<u>Strain name</u>
C43C9; C14G10	1	-	MT4302
W07H6; C14G10	3	-	MT4299
		-	MT4300
		-	MT4301
C48D1; C14G10	2	+	MT4298
		+	MT4303

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Animals injected were of genotype: *ced-1(e1735); unc-31(e929)*  
*ced-3(n717)*.

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Table 4

The expression of *ced-3(+)* transformants

Genotype	DNA injected	Average No. cell deaths in L1 head	No. Animals scored
<i>ced-1</i>	-	23	20
<i>ced-1; ced-3</i>	-	0.3	10
<i>ced-1; nIS1</i> <i>unc-31 ced-3</i>	C48D1; C14G10	16.4	20
<i>ced-1; unc-31</i> <i>ced-3; nIS1/+</i>		14.5	20
<i>ced-1; unc-31</i> <i>ced-3; nEX2</i>	C48D1; C14G10	13.2	10/14
		0	4/14
<i>ced-1; unc-31</i> <i>ced-3; nEX10</i>	C48D1-28; C14G10	12	9/10
		0	1 of 10
<i>ced-1; unc-31</i> <i>ced-3; nEX9</i>	C48D1-28; C14G10	12	10
<i>ced-1; unc-31</i> <i>ced-3; nEX11</i>	C48D1-43 C14G10	16.7	10/13
		Abnormal cell deaths	3/13
<i>ced-1; unc-31</i> <i>ced-3; nEX13</i>	pJ40; C14G10	13.75	4/4

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Table 4 continued

<i>ced-1; unc-31</i> <i>ced-3; nEX17</i>	pJ107del128, pJ107del134 C14G10	23	12/14
		0	2/14
<i>ced-1; unc-31</i> <i>ced-3; nEX18</i>	pJ107del128, pJ107del134 C14G10	12.8	9/10
		0	1/10
<i>ced-1; unc-31</i> <i>ced-3; nEX19</i>	pJ107del128, pJ107del134 G14G10	10.6	5/6
		0	1/6
<i>ced-1; unc-31</i> <i>ced-3; nEX16</i>	pJ107del112, pJ107del127 C14G10	7.8	12/12

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Alleles of the genes used are *ced-1*(e1735), *unc-31*(e928), and *ced-3*(n717).

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT

(A) NAME: Massachusetts Institute of Technology  
 (B) STREET: 77 Massachusetts Avenue  
 (C) CITY: Cambridge  
 (D) STATE OR PROVINCE: Massachusetts  
 (E) COUNTRY: U.S.A.  
 (F) POSTAL CODE: 02139

(ii) TITLE OF INVENTION: Inhibitors of Ced-3 and Related Proteins

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: diskette

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7653 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TTGTCGAATT AATATCCCTA TTATCACTTT TTCATGCTCA TCCTCGAGCG GCACGTCCTC	180
AAAGAATTGT GAGAGCAAAC GCGCTCCCAT TGACCTCCAC ACTCAGCCGC CAAAACAAAC	240
GTTCGAACAT TCGTGTGTTG TGCTCCTTTT CCGTTATCTT GCAGTCATCT TTTGTCGTTT	300
TTTTCTTTGT TCTTTTGTGTT GAACGTGTTG CTAAGCAATT ATTACATCAA TTGAAGAAAA	360
GGCTCGCCGA TTTATTGTTG CCAGAAAGAT TCTGAGATTC TCGAAGTCGA TTTTATAATA	420
TTTAACCTTG GTTTTTGCAT TGTTTCGTTT AAAAAACCA CTGTTTATGT GAAAAACGAT	480
TAGTTTACTA ATAAACTAC TTTTAAACCT TTACCTTTAC CTCACCGCTC CGTGTTTCATG	540
GCTCATAGAT TTTCGATACT CAAATCCAAA AATAAATTTA CGAGGGCAAT TAATGTGAAA	600
CAAAAACAAT CCTAAGATTT CCACATGTTT GACCTCTCCG GCACCTTCTT CCTTAGCCCC	660
ACCACTCCAT CACCTCTTTG GCGGTGTTCT TCGAAACCCA CTTAGGAAAG CAGTGTGTAT	720
CTCATTGTTG ATGCTCTTTT CGATTTTATA GCTCTTTGTC GCAATTTCAA TGCTTTAAAC	780

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AATCCAAATC GCATTATATT TGTGCATGGA GGCAAATGAC GGGGTTGGAA TCTTAGATGA	840
GATCAGGAGC TTTCAGGGTA AACGCCCCGGT TCATTTTGTA CCACATTTCA TCATTTTCCT	900
GTCGTCCTTG GTATCCTCAA CTTGTCCCGG TTTTGTTCCT GGTACACTCT TCCGTGATGC	960
CACCTGTCTC CGTCTCAATT ATCGTTTAGA AATGTGAACT GTCCAGATGG GTGACTCATA	1020
TTGCTGCTGC TACAATCCAC TTTCTTTTCT CATCGGCAGT CTTACGAGCC CATCATAAAC	1080
TTTTTTTTTC GCGAAATTTG CAATAAACCG GCCAAAACT TTCTCCAAAT TGTTACGCAA	1140
TATATACAAT CCATAAGAAT ATCTTCTCAA TGTTTATGAT TTCTTCGCAG CACTTTCTCT	1200
TCGTGTGCTA ACATCTTATT TTTATAATAT TTCCGCTAAA ATTCCGATTT TTGAGTATTA	1260
ATTTATCGTA AAATTATCAT AATAGCACCG AAACTACTA AAAATGGTAA AAGCTCCTTT	1320
TAAATCGGCT CGACATTATC GTATTAAGGA ATCACAAAAT TCTGAGAATG CGTACTGCGC	1380
AACATATTTG ACGGCAAAAT ATCTCGTAGC GAAACTACA GTAATTCTTT AAATGACTAC	1440
TGTAGCGCTT GTGTCGATTT ACGGGCTCAA TTTTGAAAA TAATTTTTTT TTTTGAATTT	1500
TGATAACCCG TAAATCGTCA CAACGCTACA GTAGTCATTT AAAGGATTAC TGTAGTTCTA	1560
GCTACGAGAT ATTTTGCGCG CCAAATATGA CTGTAATACG CATTCTCTGA ATTTTGTGTT	1620
TCCGTAATAA TTTCACAAGA TTTTGGCATT CCACTTTAAA GGCGCACAGG ATTTATTCCA	1680
ATGGGTCTCG GCACGCAAAA AGTTTGATAG ACTTTTAAAT TCTCCTTGCA TTTTAAATTC	1740
AATTACTAAA ATTTTCGTGA ATTTTCTGT TAAATTTTT AAAATCAGTT TTCTAATATT	1800
TTCCAGGCTG ACAAACAGAA ACAAAAACAC AACAAACATT TAAAAATCA GTTTTCAAAT	1860
TAAAAATAAC GATTTCTCAT TGAAAATTGT GTTTTATGTT TGCGAAAATA AAAGAGAACT	1920
GATTCAAAAC AATTTTAACA AAAAAAACC CCAAATTCG CCAGAAATCA AGATAAAAAA	1980
TTCAAGAGGG TCAAATTTT CCGATTTTAC TGAATTTTAC CTTTTTTTTT GTAGTTCAGT	2040
GCAGTTGTTG GAGTTTTTGA CGAAACTAG GAAAAAATC GATAAAAATT ACTCAAATCG	2100
AGCTGAATTT TGAGGACAAT GTTTAAAAA AACACTATT TTTCCAATAA TTCACTCAT	2160
TTTCAGACTA AATCGAAAAT CAAATCGTAC TCTGACTACG GGTCAGTAGA GAGGTCAACC	2220
ATCAGCCGAA GATGATGCGT CAAGATAGAA GGAGCTTGCT AGAGAGGAAC ATTATGATGT	2280
TCTCTAGTCA TCTAAAAGTC GATGAAATTC TCGAAGTTCT CATCGCAAAA CAAGTGTGTA	2340
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AATTGATAAT ATAAAGAATA TTTTGCAGT CATGTGGAAC GGTTCGCGAG AAGAGACGGG	2460
AGATCGTGAA AGCAGTGCAA CGACGGGGAG ATGTGGCGTT CGACGCGTTT TATGATGCTC	2520

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TTTTTAAAGT	TCGGCGCAAA	AGCAAGGGTC	TCACGGAAAA	AAGAGGCGGA	TCGTAATTTT	2640
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TTGAAGTGAA	ATATATTTTA	TTTACTGAAA	GCTCGAGTGA	TTATTTATTT	TTTAACACTA	2760
ATTTTCGTGG	CGCAAAAGGC	CATTTTGTAG	ATTTGCCGAA	AATACTTGTC	ACACACACAC	2820
ACACACATCT	CCTTCAAATA	TCCCTTTTTT	CAGTGTGAC	TCGAATGCTG	TCGAATTCGA	2880
GTGTCCAATG	TCACCGGCAA	GCCATCGTCG	GAGCCGCGCA	TTGAGCCCCG	CCGGCTACAC	2940
TTCACCGACC	CGAGTTCACC	GTGACAGCGT	CTCTTCAGTG	TCATCATTCA	CTTCTTATCA	3000
GGATATCTAC	TCAAGAGCAA	GATCTCGTTC	TCGATCGCGT	GCACTTCATT	CATCGGATCG	3060
ACACAATTAT	TCATCTCCTC	CAGTCAACGC	ATTTCCAGC	CAACCTTGTA	TGTTGATGCG	3120
AACACTAAAT	TCTGAGAATG	CGCATTACTC	AACATATTTG	ACGCGCAAAT	ATCTCGTAGC	3180
GAAAAATACA	GTAACCCCTT	AAATGACTAT	TGTAGTGTG	ATTTACGGGC	TCGATTTTCG	3240
AAACGAATAT	ATGCTCGAAT	TGTGACAACG	AATTTTAATT	TGTCATTTTT	GTGTTTCTT	3300
TTGATATTTT	TGATCAATTA	ATAAATTATT	TCCGTAAACA	GACACCAGCG	CTACAGTACT	3360
CTTTTAAAGA	GTTACAGTAG	TTTTCGCTTC	AAGATATTTT	GAAAAGAATT	TTAAACATTT	3420
TGAAAAAAA	TCATCTAACA	TGTGCCAAAA	CGCTTTTTTC	AAGTTTCGCA	GATTTTTTGA	3480
TTTTTTTCAT	TCAAGATATG	CTTATTAACA	CATATAATTA	TCATTAATGT	GAATTTCTTG	3540
TAGAAATTTT	GGGCTTTTCG	TTCTAGTATG	CTCTACTTTT	GAAATTGCTC	AACGAAAAAA	3600
TCATGTGGTT	TGTTCATATG	AATGACGAAA	AATAGCAATT	TTTTATATAT	TTTCCCCTAT	3660
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GCGTCTCTCG	TCTTCAGCAT	GTGAAATGGG	ATCTCGGTCG	ATGTAAAAAA	ATGTCGAATA	3840
ATGTAAAAAA	TGCATGCGTT	TTTTTACACT	TTTCTGCACA	AATGAATAGG	GGGAAAATGT	3900
ATTAAAATAC	ATTTTTTGTA	TTTTTCAACA	TCACATGATT	AACCCCATTA	TTTTTTCGTT	3960
GAGCAACTTA	AAAAGTAGAG	AATATTAGAG	CGAAAACCAA	AATTTCTTCA	AGATATTACC	4020
TTTATTGATA	ATTATAGATG	TTAATAAGCA	TATCTTGAAT	GAAAGTCAGC	AAAAATATGT	4080
GCGAAACACC	TGAAAAAAT	CAAAAATTCT	GCGAAAATTG	AAAAAATGCA	TTAAAATACA	4140
TTTTTGCAAT	TTTCTACATC	ACATGAATGT	AGAAAATTAA	AAGGGAAATC	AAAATTCTA	4200
GAGGATATAA	TTGAATGAAA	CATTGCGAAA	TTAAAATGTG	CGAAACGTCA	AAAAAGAGGA	4260



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AATTTGGGTA	TCAAATCGA	TCCTAAAACC	AACACATTTT	AGCATCCGCC	AACTCTTCAT	4320
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CTGGACCAAC	TCAATACATA	TTCCATGAAG	AGGATATGAA	CTTGTCGAT	GCACCAACCA	4440
TAAGCCGTGT	TTTCGACGAG	AAAACCATGT	ACAGAACTT	CTCGAGTCCT	CGTGGAATGT	4500
GCCTCATCAT	AAATAATGAA	CACTTTGAGC	AGATGCCAAC	ACGGAATGGT	ACCAAGGCCG	4560
ACAAGGACAA	TCTTACCAAT	TTGTTTCAGAT	GCATGGGCTA	TACGGTTATT	TGCAAGGACA	4620
ATCTGACGGG	AAGGGTACGG	CGAAATTATA	TTACCCAAAC	GCGAAATTTG	CCATTTTGCG	4680
CCGAAAATGT	GGCGCCCGGT	CTCGACACGA	CAATTTGTGT	TAAATGCAA	AATGTATAAT	4740
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ACCGTTTGTA	AATTTTCAGA	CAATTTTCCG	CATACAAAAC	TTGATAGCAC	GAAATCAATT	4920
TTCTGAATTT	TCAAATTTAT	CCAAAATGTC	ACAATTTAAA	ATTTGTGAAA	ATTGGCAAAC	4980
GGTGTTCAT	TATGAAATGT	ATTTTTAAAA	ACTTTAAAAA	CCACTCCGGA	AAAGCAATAA	5040
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CAAAATTTGA	AAAAATCATG	AAGGATTTAG	AAAAGTTTTA	TAACATTTTT	TCTAGATTTT	5160
TCAAATTTT	TTTAAACAAA	TCGAGAAAAA	GAGAATGAAA	AATCGATTTT	AAAAATATCC	5220
ACAGCTTCGA	GAGTTTGAAA	TTACAGTACT	CCTTAAAGGC	GCACACCCCA	TTTGCATTGG	5280
ACCAAAAATT	TGTCGTGTCG	AGACCAGGTA	CCGTAGTTTT	TGTCGCAAAA	ATTGCACCAT	5340
TGGACAATAA	ACCTTCCTAA	TCACCAAAAA	GTAAATTTGA	AATCTTCGAA	AAGCCAAAAA	5400
ATTCAAAAAA	AAAGTCGAAT	TTCGATTTTT	TTTTTGTTTT	TTTGGTCCCA	AAAACCAAAA	5460
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ACGAATCACA	CGGAGATTCT	GCGATACTCG	TGATTCTATC	ACACGGAGAA	GAGAATGTGA	5640
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CAAATGCTCC	CCGTCTGGCG	AATAAGCCGA	AAATCGTTTT	TGTGCAGGCT	TGTCGAGGCG	5760
GTTCGTTTTT	TATTTTAATT	TTAATATAAA	TATTTTAAAT	AAATTCATTT	TCAGAACGTC	5820
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GATGGGACAA	TCGAGACGGG	CCATTGTTCA	ATTTTCTTGG	ATGTGTGCGG	CCGCAAGTTC	5940
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AAATTAAAAA	TTGATTTTTT	CAATTTTTTC	GAAAAATATT	CCGATTATTT	TATATTCTTT	6240
GGAGCGAAAG	CCCCGTCCTG	TAAACATTTT	TAAATGATAA	TTAATAAATT	TTTGCAGCAA	6300
GTGTGGAGAA	AGAAGCCGAG	CCAAGCTGAC	ATTCTGATTC	GATACGCAAC	GACAGCTCAA	6360
TATGTTTCGT	GGAGAAACAG	TGCTCGTGGA	TCATGGTTCA	TTCAAGCCGT	CTGTGAAGTG	6420
TTCTCGACAC	ACGCAAAGGA	TATGGATGTT	GTTGAGCTGC	TGACTGAAGT	CAATAAGAAG	6480
GTCGCTTG TG	GATTTTCAGAC	ATCACAGGGA	TCGAATATTT	TGAAACAGAT	GCCAGAGGTA	6540
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TTTGCAAGCC	TGCCGCGCGT	CAACCTAGAA	TTTtagTTTT	TAGCTAAAAT	GATTGATTTT	6660
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ACACGGTTGG	CGCGCGGCAA	GTTTGCAAAA	CGACGCTCCG	CCTCTTTTTC	TGTGCGGCTT	6960
GAAAACAAGG	GATCGGTTTA	GATTTTTCCC	CAAAATTTAA	ATTAAATTTT	AGATGACATC	7020
CCGCCTGCTC	AAAAAGTTCT	ACTTTTGGCC	GGAAGCACGA	AACTCTGCCG	TCTAAAATTC	7080
ACTCGTGATT	CATTGCCCAA	TTGATAATTG	TCTGTATCTT	CTCCCCCAGT	TCTCTTTCGC	7140
CCAATTAGTT	TAAAACCATG	TGTATATTGT	TATCCTATAC	TCATTTCACT	TTATCATTCT	7200
ATCATTTCTC	TTCCCATTTT	CACACATTTT	CATTTCTCTA	CGATAATCTA	AAATTATGAC	7260
GTTTGTGTCT	CGAACGCATA	ATAATTTTAA	TAACTCGTTT	TGAATTTGAT	TAGTTGTTGT	7320
GCCCAGTATA	TATGTATGTA	CTATGCTTCT	ATCAACAAAA	TAGTTTCATA	GATCATCACC	7380
CCAACCCAC	CAACCTACCG	TACCATATTC	ATTTTGGCCG	GGAATCAATT	TCGATTAATT	7440
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TCTCCCGTGC	CGGAATGCCT	CCCGGCCTTT	TAAAGTTCCG	AACATTTGGC	AATTATGTAT	7560
AAATTTGTAG	GTCCCCCCCA	TCATTTCCCG	CCCATCATCT	CAAATTGCAT	TCTTTTTTCG	7620
CCGTGATATC	CCGATTCTGG	TCAGCAAAGA	TCT			7653

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## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 503 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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          20           25           30
Lys Gln Val Leu Asn Ser Asp Asn Gly Asp Met Ile Asn Ser Cys Gly
          35           40           45
Thr Val Arg Glu Lys Arg Arg Glu Ile Val Lys Ala Val Gln Arg Arg
          50           55           60
Gly Asp Val Ala Phe Asp Ala Phe Tyr Asp Ala Leu Arg Ser Thr Gly
65           70           75           80
His Glu Gly Leu Ala Glu Val Leu Glu Pro Leu Ala Arg Ser Val Asp
          85           90           95
Ser Asn Ala Val Glu Phe Glu Cys Pro Met Ser Pro Ala Ser His Arg
          100          105          110
Arg Ser Arg Ala Leu Ser Pro Ala Gly Tyr Thr Ser Pro Thr Arg Val
          115          120          125
His Arg Asp Ser Val Ser Ser Val Ser Ser Phe Thr Ser Tyr Gln Asp
          130          135          140
Ile Tyr Ser Arg Ala Arg Ser Arg Ser Arg Ser Arg Ala Leu His Ser
145          150          155          160
Ser Asp Arg His Asn Tyr Ser Ser Pro Pro Val Asn Ala Phe Pro Ser
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Gln Pro Ser Ser Ala Asn Ser Ser Phe Thr Gly Cys Ser Ser Leu Gly
          180          185          190
Tyr Ser Ser Ser Arg Asn Arg Ser Phe Ser Lys Ala Ser Gly Pro Thr
          195          200          205
Gln Tyr Ile Phe His Glu Glu Asp Met Asn Phe Val Asp Ala Pro Thr
          210          215          220
Ile Ser Arg Val Phe Asp Glu Lys Thr Met Tyr Arg Asn Phe Ser Ser
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Pro Arg Gly Met Cys Leu Ile Ile Asn Asn Glu His Phe Glu Gln Met
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Pro Thr Arg Asn Gly Thr Lys Ala Asp Lys Asp Asn Leu Thr Asn Leu  
                   260                  265                  270  
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                   275                  280                  285  
 Arg Gly Met Leu Leu Thr Ile Arg Asp Phe Ala Lys His Glu Ser His  
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 Gly Asp Ser Ala Ile Leu Val Ile Leu Ser His Gly Glu Glu Asn Val  
                   305                  310                  315                  320  
 Ile Ile Gly Val Asp Asp Ile Pro Ile Ser Thr His Glu Ile Tyr Asp  
                   325                  330                  335  
 Leu Leu Asn Ala Ala Asn Ala Pro Arg Leu Ala Asn Lys Pro Lys Ile  
                   340                  345                  350  
 Val Phe Val Gln Ala Cys Arg Gly Glu Arg Arg Asp Asn Gly Phe Pro  
                   355                  360                  365  
 Val Leu Asp Ser Val Asp Gly Val Pro Ala Phe Leu Arg Arg Gly Trp  
                   370                  375                  380  
 Asp Asn Arg Asp Gly Pro Leu Phe Asn Phe Leu Gly Cys Val Arg Pro  
                   385                  390                  395                  400  
 Gln Val Gln Gln Val Trp Arg Lys Lys Pro Ser Gln Ala Asp Ile Leu  
                   405                  410                  415  
 Ile Arg Tyr Ala Thr Thr Ala Gln Tyr Val Ser Trp Arg Asn Ser Ala  
                   420                  425                  430  
 Arg Gly Ser Trp Phe Ile Gln Ala Val Cys Glu Val Phe Ser Thr His  
                   435                  440                  445  
 Ala Lys Asp Met Asp Val Val Glu Leu Leu Thr Glu Val Asn Lys Lys  
                   450                  455                  460  
 Val Ala Cys Gly Phe Gln Thr Ser Gln Gly Ser Asn Ile Leu Lys Gln  
                   465                  470                  475                  480  
 Met Pro Glu Met Thr Ser Arg Leu Leu Lys Lys Phe Tyr Phe Trp Pro  
                   485                  490                  495  
 Glu Ala Arg Asn Ser Ala Val  
                   500

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1373 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

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## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 18..1232

(D) OTHER INFORMATION: /product= "human interleukin-1 $\beta$  convertase"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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1 5 10	
CTG TTT ATC CGT TCC ATG GGT GAA GGT ACA ATA AAT GGC TTA CTG GAT	98
Leu Phe Ile Arg Ser Met Gly Glu Gly Thr Ile Asn Gly Leu Leu Asp	
15 20 25	
GAA TTA TTA CAG ACA AGG GTG CTG AAC AAG GAA GAG ATG GAG AAA GTA	146
Glu Leu Leu Gln Thr Arg Val Leu Asn Lys Glu Glu Met Glu Lys Val	
30 35 40	
AAA CGT GAA AAT GCT ACA GTT ATG GAT AAG ACC CGA GCT TTG ATT GAC	194
Lys Arg Glu Asn Ala Thr Val Met Asp Lys Thr Arg Ala Leu Ile Asp	
45 50 55	
TCC GTT ATT CCG AAA GGG GCA CAG GCA TGC CAA ATT TGC ATC ACA TAC	242
Ser Val Ile Pro Lys Gly Ala Gln Ala Cys Gln Ile Cys Ile Thr Tyr	
60 65 70 75	
ATT TGT GAA GAA GAC AGT TAC CTG GCA GGG ACG CTG GGA CTC TCA GCA	290
Ile Cys Glu Glu Asp Ser Tyr Leu Ala Gly Thr Leu Gly Leu Ser Ala	
80 85 90	
GAT CAA ACA TCT GGA AAT TAC CTT AAT ATG CAA GAC TCT CAA GGA GTA	338
Asp Gln Thr Ser Gly Asn Tyr Leu Asn Met Gln Asp Ser Gln Gly Val	
95 100 105	
CTT TCT TCC TTT CCA GCT CCT CAG GCA GTG CAG GAC AAC CCA GCT ATG	386
Leu Ser Ser Phe Pro Ala Pro Gln Ala Val Gln Asp Asn Pro Ala Met	
110 115 120	
CCC ACA TCC TCA GGC TCA GAA GGG AAT GTC AAG CTT TGC TCC CTA GAA	434
Pro Thr Ser Ser Gly Ser Glu Gly Asn Val Lys Leu Cys Ser Leu Glu	
125 130 135	
GAA GCT CAA AGG ATA TGG AAA CAA AAG TCG GCA GAG ATT TAT CCA ATA	482
Glu Ala Gln Arg Ile Trp Lys Gln Lys Ser Ala Glu Ile Tyr Pro Ile	
140 145 150 155	
ATG GAC AAG TCA AGC CGC ACA CGT CTT GCT CTC ATT ATC TGC AAT GAA	530
Met Asp Lys Ser Ser Arg Thr Arg Leu Ala Leu Ile Ile Cys Asn Glu	
160 165 170	
GAA TTT GAC AGT ATT CCT AGA AGA ACT GGA GCT GAG GTT GAC ATC ACA	578
Glu Phe Asp Ser Ile Pro Arg Arg Thr Gly Ala Glu Val Asp Ile Thr	
175 180 185	

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GGC ATG ACA ATG CTG CTA CAA AAT CTG GGG TAC AGC GTA GAT GTG AAA Gly Met Thr Met Leu Leu Gln Asn Leu Gly Tyr Ser Val Asp Val Lys 190 195 200	626
AAA AAT CTC ACT GCT TCG GAC ATG ACT ACA GAG CTG GAG GCA TTT GCA Lys Asn Leu Thr Ala Ser Asp Met Thr Thr Glu Leu Glu Ala Phe Ala 205 210 215	674
CAC CGC CCA GAG CAC AAG ACC TCT GAC AGC ACG TTC CTG GTG TTC ATG His Arg Pro Glu His Lys Thr Ser Asp Ser Thr Phe Leu Val Phe Met 220 225 230 235	722
TCT CAT GGT ATT CGG GAA GGC ATT TGT GGG AAG AAA CAC TCT GAG CAA Ser His Gly Ile Arg Glu Gly Ile Cys Gly Lys Lys His Ser Glu Gln 240 245 250	770
GTC CCA GAT ATA CTA CAA CTC AAT GCA ATC TTT AAC ATG TTG AAT ACC Val Pro Asp Ile Leu Gln Leu Asn Ala Ile Phe Asn Met Leu Asn Thr 255 260 265	818
AAG AAC TGC CCA AGT TTG AAG GAC AAA CCG AAG GTG ATC ATC ATC CAG Lys Asn Cys Pro Ser Leu Lys Asp Lys Pro Lys Val Ile Ile Ile Gln 270 275 280	866
GCC TGC CGT GGT GAC AGC CCT GGT GTG GTG TGG TTT AAA GAT TCA GTA Ala Cys Arg Gly Asp Ser Pro Gly Val Val Trp Phe Lys Asp Ser Val 285 290 295	914
GGA GTT TCT GGA AAC CTA TCT TTA CCA ACT ACA GAA GAG TTT GAG GAT Gly Val Ser Gly Asn Leu Ser Leu Pro Thr Thr Glu Glu Phe Glu Asp 300 305 310 315	962
GAT GCT ATT AAG AAA GCC CAC ATA GAG AAG GAT TTT ATC GCT TTC TGC Asp Ala Ile Lys Lys Ala His Ile Glu Lys Asp Phe Ile Ala Phe Cys 320 325 330	1010
TCT TCC ACA CCA GAT AAT GTT TCT TGG AGA CAT CCC ACA ATG GGC TCT Ser Ser Thr Pro Asp Asn Val Ser Trp Arg His Pro Thr Met Gly Ser 335 340 345	1058
GTT TTT ATT GGA AGA CTC ATT GAA CAT ATG CAA GAA TAT GCC TGT TCC Val Phe Ile Gly Arg Leu Ile Glu His Met Gln Glu Tyr Ala Cys Ser 350 355 360	1106
TGT GAT GTG GAG GAA ATT TTC CGC AAG GTT CGA TTT TCA TTT GAG CAG Cys Asp Val Glu Glu Ile Phe Arg Lys Val Arg Phe Ser Phe Glu Gln 365 370 375	1154
CCA GAT GGT AGA GCG CAG ATG CCC ACC ACT GAA AGA GTG ACT TTG ACA Pro Asp Gly Arg Ala Gln Met Pro Thr Thr Glu Arg Val Thr Leu Thr 380 385 390 395	1202
AGA TGT TTC TAC CTC TTC CCA GGA CAT TAAATAAGG AAAGTGTATG Arg Cys Phe Tyr Leu Phe Pro Gly His 400 405	1249
AATGTCTGCG GGCAGGAAGT GAAGAGATCG TTCTGTAAAA GGTTTTTGA ATTATGTCTG	1309

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CTGAATAATA AACTTTTTTTT GAAATAATAA ATCTGGTAGA AAAATGAAA AAAAAAAAAA 1369  
 AAAA 1373

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 404 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Asp	Lys	Val	Leu	Lys	Glu	Lys	Arg	Lys	Leu	Phe	Ile	Arg	Ser	
1				5					10					15		
Met	Gly	Glu	Gly	Thr	Ile	Asn	Gly	Leu	Leu	Asp	Glu	Leu	Leu	Gln	Thr	
			20					25						30		
Arg	Val	Leu	Asn	Lys	Glu	Glu	Met	Glu	Lys	Val	Lys	Arg	Glu	Asn	Ala	
		35					40					45				
Thr	Val	Met	Asp	Lys	Thr	Arg	Ala	Leu	Ile	Asp	Ser	Val	Ile	Pro	Lys	
		50				55					60					
Gly	Ala	Gln	Ala	Cys	Gln	Ile	Cys	Ile	Thr	Tyr	Ile	Cys	Glu	Glu	Asp	
	65				70					75					80	
Ser	Tyr	Leu	Ala	Gly	Thr	Leu	Gly	Leu	Ser	Ala	Asp	Gln	Thr	Ser	Gly	
				85					90					95		
Asn	Tyr	Leu	Asn	Met	Gln	Asp	Ser	Gln	Gly	Val	Leu	Ser	Ser	Phe	Pro	
			100					105						110		
Ala	Pro	Gln	Ala	Val	Gln	Asp	Asn	Pro	Ala	Met	Pro	Thr	Ser	Ser	Gly	
			115				120					125				
Ser	Glu	Gly	Asn	Val	Lys	Leu	Cys	Ser	Leu	Glu	Glu	Ala	Gln	Arg	Ile	
	130					135					140					
Trp	Lys	Gln	Lys	Ser	Ala	Glu	Ile	Tyr	Pro	Ile	Met	Asp	Lys	Ser	Ser	
	145				150					155					160	
Arg	Thr	Arg	Leu	Ala	Leu	Ile	Ile	Cys	Asn	Glu	Glu	Phe	Asp	Ser	Ile	
				165				170						175		
Pro	Arg	Arg	Thr	Gly	Ala	Glu	Val	Asp	Ile	Thr	Gly	Met	Thr	Met	Leu	
			180					185					190			
Leu	Gln	Asn	Leu	Gly	Tyr	Ser	Val	Asp	Val	Lys	Lys	Asn	Leu	Thr	Ala	
		195					200					205				
Ser	Asp	Met	Thr	Thr	Glu	Leu	Glu	Ala	Phe	Ala	His	Arg	Pro	Glu	His	
	210					215					220					

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Lys Thr Ser Asp Ser Thr Phe Leu Val Phe Met Ser His Gly Ile Arg  
 225 230 235 240  
 Glu Gly Ile Cys Gly Lys Lys His Ser Glu Gln Val Pro Asp Ile Leu  
 245 250 255  
 Gln Leu Asn Ala Ile Phe Asn Met Leu Asn Thr Lys Asn Cys Pro Ser  
 260 265 270  
 Leu Lys Asp Lys Pro Lys Val Ile Ile Ile Gln Ala Cys Arg Gly Asp  
 275 280 285  
 Ser Pro Gly Val Val Trp Phe Lys Asp Ser Val Gly Val Ser Gly Asn  
 290 295 300  
 Leu Ser Leu Pro Thr Thr Glu Glu Phe Glu Asp Asp Ala Ile Lys Lys  
 305 310 315 320  
 Ala His Ile Glu Lys Asp Phe Ile Ala Phe Cys Ser Ser Thr Pro Asp  
 325 330 335  
 Asn Val Ser Trp Arg His Pro Thr Met Gly Ser Val Phe Ile Gly Arg  
 340 345 350  
 Leu Ile Glu His Met Gln Glu Tyr Ala Cys Ser Cys Asp Val Glu Glu  
 355 360 365  
 Ile Phe Arg Lys Val Arg Phe Ser Phe Glu Gln Pro Asp Gly Arg Ala  
 370 375 380  
 Gln Met Pro Thr Thr Glu Arg Val Thr Leu Thr Arg Cys Phe Tyr Leu  
 385 390 395 400  
 Phe Pro Gly His

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 505 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: unsure
- (B) LOCATION: at every Xaa

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Met Arg Gln Asp Arg Trp Leu Leu Glu Arg Asn Ile Leu Glu Phe  
 1 5 10 15  
 Ser Ser Lys Leu Gln Ala Asp Leu Ile Leu Asp Val Leu Ile Ala Lys  
 20 25 30



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Gln Val Leu Asn Ser Asp Asn Gly Asp Val Ile Asn Ser Cys Arg Thr  
 35 40 45  
 Glu Arg Asp Asn Glu Lys Glu Ile Val Lys Ala Val Gln Arg Arg Gly  
 50 55 60  
 Asp Glu Ala Phe Asp Ala Phe Tyr Asp Ala Leu Arg Asp Thr Gly His  
 65 70 75 80  
 Asn Asp Leu Ala Asp Val Leu Met Pro Leu Ser Arg Pro Xaa Xaa Xaa  
 85 90 95  
 Asn Pro Val Pro Met Glu Cys Pro Met Ser Pro Ser Ser His Arg Arg  
 100 105 110  
 Ser Arg Ala Leu Ser Pro Pro Xaa Tyr Ala Ser Pro Thr Arg Val His  
 115 120 125  
 Arg Asp Ser Ile Ser Ser Val Ser Ser Phe Thr Ser Thr Tyr Gln Asp  
 130 135 140  
 Val Tyr Ser Arg Ala Arg Ser Ser Ser Arg Ser Ser Arg Pro Leu Gln  
 145 150 155 160  
 Ser Ser Asp Arg His Asn Tyr Met Ser Ala Ala Thr Ser Phe Pro Ser  
 165 170 175  
 Gln Pro Xaa Ser Ala Asn Ser Ser Phe Thr Gly Cys Ala Ser Leu Gly  
 180 185 190  
 Tyr Ser Ser Ser Arg Asn Arg Ser Phe Ser Lys Thr Ser Ala Gln Ser  
 195 200 205  
 Gln Tyr Ile Phe His Glu Glu Asp Met Asn Tyr Val Asp Ala Pro Thr  
 210 215 220  
 Ile His Arg Val Phe Asp Glu Lys Thr Met Tyr Arg Asn Phe Ser Ser  
 225 230 235 240  
 Pro Arg Gly Leu Cys Leu Ile Ile Asn Asn Glu His Phe Glu Gln Met  
 245 250 255  
 Pro Thr Arg Asn Gly Thr Lys Ala Asp Lys Asp Asn Leu Thr Asn Ile  
 260 265 270  
 Phe Arg Cys Met Gly Tyr Thr Val Ile Cys Lys Asp Asn Leu Thr Gly  
 275 280 285  
 Arg Glu Met Leu Ser Thr Ile Arg Ser Phe Gly Arg Asn Asp Met His  
 290 295 300  
 Gly Asp Ser Ala Ile Leu Val Ile Leu Ser His Gly Glu Xaa Asn Val  
 305 310 315 320  
 Ile Ile Gly Val Asp Asp Val Ser Val Asn Val His Glu Ile Tyr Asp  
 325 330 335  
 Leu Leu Asn Ala Ala Asn Ala Pro Arg Leu Ala Asn Lys Pro Lys Leu

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340	345	350
Val Phe Val Gln Ala Cys Arg Gly Glu Arg Arg Asp Asn Gly Phe Pro		
355	360	365
Val Leu Asp Ser Val Asp Gly Val Pro Ser Leu Ile Arg Arg Gly Trp		
370	375	380
Asp Asn Arg Asp Gly Pro Leu Phe Asn Phe Leu Gly Cys Val Arg Pro		
385	390	395
Gln Val Gln Gln Val Trp Arg Lys Lys Pro Ser Gln Ala Asp Met Leu		
405	410	415
Ile Ala Tyr Ala Thr Thr Ala Gln Tyr Val Ser Trp Arg Asn Ser Ala		
420	425	430
Arg Gly Ser Trp Phe Ile Gln Ala Val Cys Glu Val Phe Ser Leu His		
435	440	445
Ala Lys Asp Met Asp Val Val Glu Leu Leu Thr Glu Val Asn Lys Lys		
450	455	460
Val Ala Cys Gly Phe Gln Thr Ser Gln Gly Ser Asn Ile Leu Lys Gln		
465	470	475
Met Pro Glu Leu Thr Ser Arg Leu Leu Lys Lys Phe Tyr Phe Trp Pro		
485	490	495
Glu Asp Arg Gly Arg Asn Ser Ala Val		
500	505	

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 480 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (ix) FEATURE:  
 (A) NAME/KEY: unsure  
 (B) LOCATION: at every Xaa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Thr	Val	Ser	Ile	Ser	Leu	Ile	Ile	Ala	Arg	Gln	Val	Leu	Asn	Ser	Asp
1				5					10					15	
Asn	Xaa	Xaa	Met	Ile	Asn	Ser	Cys	Gly	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			20					25					30		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			35					40					45		

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 50 55 60  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 65 70 75 80  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 85 90 95  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 100 105 110  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Thr Ser  
 115 120 125  
 Arg Ser Ser Arg Pro Leu His Thr Ser Asp Arg His Asn Tyr Val Ser  
 130 135 140  
 Pro Ser Asn Ser Phe Gln Ser Gln Pro Ala Ser Ala Asn Ser Ser Phe  
 145 150 155 160  
 Thr Gly Ser Ser Ser Leu Gly Tyr Ser Ser Ser Arg Thr Arg Ser Tyr  
 165 170 175  
 Ser Lys Ala Ser Ala His Ser Gln Tyr Ile Phe His Glu Glu Asp Met  
 180 185 190  
 Asn Tyr Val Asp Ala Pro Thr Ile His Arg Val Phe Asp Glu Lys Thr  
 195 200 205  
 Met Tyr Arg Asn Phe Ser Thr Pro Arg Gly Leu Cys Leu Ile Ile Asn  
 210 215 220  
 Asn Glu His Phe Glu Gln Met Pro Thr Arg Asn Gly Thr Lys Pro Asp  
 225 230 235 240  
 Lys Asp Asn Ile Ser Asn Leu Phe Arg Cys Met Gly Tyr Ile Val His  
 245 250 255  
 Cys Lys Asp Asn Leu Thr Gly Arg Xaa Met Met Leu Thr Ile Arg Asp  
 260 265 270  
 Phe Ala Lys Asn Glu Thr His Gly Asp Ser Ala Ile Leu Val Ile Xaa  
 275 280 285  
 Ser His Gly Glu Glu Asn Val Ile Ile Gly Val Asp Asp Val Ser Val  
 290 295 300  
 Asn Val His Glu Ile Tyr Xaa Leu Leu Asn Ala Ala Asn Ala Pro Arg  
 305 310 315 320  
 Leu Ala Asn Lys Pro Lys Leu Val Phe Val Gln Ala Cys Arg Gly Glu  
 325 330 335  
 Arg Arg Asp Val Gly Phe Pro Val Leu Asp Ser Val Asp Gly Val Pro  
 340 345 350

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Ala	Leu	Ile	Arg	Arg	Gly	Trp	Asp	Lys	Gly	Asp	Gly	Pro	Xaa	Xaa	Asn
	355						360					365			
Phe	Leu	Gly	Cys	Val	Arg	Pro	Gln	Ala	Gln	Gln	Val	Trp	Arg	Lys	Lys
	370					375					380				
Pro	Ser	Gln	Ala	Asp	Ile	Leu	Ile	Ala	Tyr	Ala	Thr	Thr	Ala	Gln	Tyr
385					390				395						400
Val	Ser	Trp	Arg	Asn	Ser	Ala	Arg	Gly	Ser	Trp	Phe	Ile	Gln	Ala	Val
			405						410					415	
Cys	Glu	Val	Phe	Ser	Leu	His	Ala	Lys	Asp	Met	Asp	Val	Val	Glu	Leu
			420					425					430		
Leu	Thr	Glu	Val	Asn	Lys	Lys	Val	Ala	Cys	Gly	Phe	Gln	Thr	Ser	Gln
	435						440					445			
Gly	Ala	Asn	Ile	Leu	Lys	Gln	Met	Pro	Xaa	Leu	Thr	Ser	Arg	Leu	Leu
	450					455					460				
Lys	Lys	Phe	Tyr	Phe	Trp	Pro	Glu	Asp	Arg	Asn	Arg	Ser	Ser	Ala	Val
465					470					475					480

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCATCGACTT TTAGATGACT AGAGAACATC

30

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTGCACTGC TTTCACGATC TCCCGTCTCT

30

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTTTAATTAC CCAAGTTTGA G

21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGTTTAAACC AGTTACTCAA G

21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGGTGACAT TGGACACTC

19

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACTATTCAAC ACTTG

15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 171 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Leu	Thr	Val	Gln	Val	Tyr	Arg	Thr	Ser	Gln	Lys	Cys	Ser	Ser	Ser
1				5					10					15	

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Lys His Val Val Glu Val Leu Leu Asp Pro Leu Gly Thr Ser Phe Cys  
                   20                                  25                                  30  
 Ser Leu Leu Pro Pro Pro Leu Leu Leu Tyr Glu Thr Asp Arg Gly Val  
                   35                                  40                                  45  
 Asp Gln Gln Asp Gly Lys Asn His Thr Gln Ser Pro Gly Cys Glu Glu  
                   50                                  55                                  60  
 Ser Asp Ala Gly Lys Glu Glu Leu Met Lys Met Arg Leu Pro Thr Arg  
                   65                                  70                                  75                                  80  
 Ser Asp Met Ile Cys Gly Tyr Ala Cys Leu Lys Gly Asn Ala Ala Met  
                                   85                                  90                                  95  
 Arg Asn Thr Lys Arg Gly Ser Trp Tyr Ile Glu Ala Leu Thr Gln Val  
                                   100                                  105                                  110  
 Phe Ser Glu Arg Ala Cys Asp Met His Val Ala Asp Met Leu Val Lys  
                   115                                  120                                  125  
 Val Asn Ala Leu Ile Lys Glu Arg Glu Gly Tyr Ala Pro Gly Thr Glu  
                   130                                  135                                  140  
 Phe His Arg Cys Lys Glu Met Ser Glu Tyr Cys Ser Thr Leu Cys Gln  
                   145                                  150                                  155                                  160  
 Gln Leu Tyr Leu Phe Pro Gly Tyr Pro Pro Thr  
                                   165                                  170

## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 402 amino acids  
     (B) TYPE: amino acid  
     (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ala Asp Lys Ile Leu Arg Ala Lys Arg Lys Gln Phe Ile Asn Ser  
   1                  5                                  10                                  15  
 Val Ser Ile Gly Thr Ile Asn Gly Leu Leu Asp Glu Leu Leu Glu Lys  
                   20                                  25                                  30  
 Arg Val Leu Asn Gln Glu Glu Met Asp Lys Ile Lys Leu Ala Asn Ile  
                   35                                  40                                  45  
 Thr Ala Met Asp Lys Ala Arg Asp Leu Cys Asp His Val Ser Lys Lys  
                   50                                  55                                  60  
 Gly Pro Gln Ala Ser Gln Ile Phe Ile Thr Tyr Ile Cys Asn Glu Asp  
                   65                                  70                                  75                                  80

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Cys Tyr Leu Ala Gly Ile Leu Glu Leu Gln Ser Ala Pro Ser Ala Glu  
 85 90 95  
 Thr Phe Val Ala Thr Glu Asp Ser Lys Gly Gly His Pro Ser Ser Ser  
 100 105 110  
 Glu Thr Lys Glu Glu Gln Asn Lys Glu Asp Gly Thr Phe Pro Gly Leu  
 115 120 125  
 Thr Gly Thr Leu Lys Phe Cys Pro Leu Glu Lys Ala Gln Lys Leu Trp  
 130 135 140  
 Lys Glu Asn Pro Ser Glu Ile Tyr Pro Ile Met Asn Thr Thr Thr Arg  
 145 150 155 160  
 Thr Arg Leu Ala Leu Ile Ile Cys Asn Thr Glu Phe Gln His Leu Ser  
 165 170 175  
 Pro Arg Val Gly Ala Gln Val Asp Leu Arg Glu Met Lys Leu Leu Leu  
 180 185 190  
 Glu Asp Leu Gly Tyr Thr Val Lys Val Lys Glu Asn Leu Thr Ala Leu  
 195 200 205  
 Glu Met Val Lys Glu Val Lys Glu Phe Ala Ala Cys Pro Glu His Lys  
 210 215 220  
 Thr Ser Asp Ser Thr Phe Leu Val Phe Met Ser His Gly Ile Gln Glu  
 225 230 235 240  
 Gly Ile Cys Gly Thr Thr Tyr Ser Asn Glu Val Ser Asp Ile Leu Lys  
 245 250 255  
 Val Asp Thr Ile Phe Gln Met Met Asn Thr Leu Lys Cys Pro Ser Leu  
 260 265 270  
 Lys Asp Lys Pro Lys Val Ile Ile Ile Gln Ala Cys Arg Gly Glu Lys  
 275 280 285  
 Gln Gly Val Val Leu Leu Lys Asp Ser Val Arg Asp Ser Glu Glu Asp  
 290 295 300  
 Phe Leu Thr Asp Ala Ile Phe Glu Asp Asp Gly Ile Lys Lys Ala His  
 305 310 315 320  
 Ile Glu Lys Asp Phe Ile Ala Phe Cys Ser Ser Thr Pro Asp Asn Val  
 325 330 335  
 Ser Trp Arg His Pro Val Arg Gly Ser Leu Phe Ile Glu Ser Leu Ile  
 340 345 350  
 Lys His Met Lys Glu Tyr Ala Trp Ser Cys Asp Leu Glu Asp Ile Phe  
 355 360 365  
 Arg Lys Val Arg Phe Ser Phe Glu Gln Pro Glu Phe Arg Leu Gln Met  
 370 375 380

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Pro	Thr	Ala	Asp	Arg	Val	Thr	Leu	Thr	Lys	Arg	Phe	Tyr	Leu	Phe	Pro
385					390					395					400

Gly His



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CLAIMS

1. An inhibitor of the activity of the *ced-3* gene,  
comprising a portion of the *ced-3* gene.
2. The inhibitor of Claim 1, wherein the gene portion  
5 is a portion of the nucleotide sequence of Figure 3  
(SEQ ID NO:1), selected from the group consisting  
of:
  - a) nucleotides 1 to approximately 5850;
  - b) nucleotides 1 to approximately 3020; and
  - 10 c) an inhibitory subportion (a) and (b).
3. The inhibitor of Claim 1, wherein the gene portion  
encodes an amino acid sequence of the Ced-3 protein  
shown in Figures 6A-B (SEQ ID NO:2), selected from  
the group consisting of:
  - 15 a) amino acids 1 to approximately 372;
  - b) amino acids 1 to approximately 149; and
  - c) an inhibitory subportion of (a) and (b).
4. The inhibitor of Claim 1, further comprising a  
heterologous structural gene fused 3' of the gene  
20 portion.
5. The inhibitor of Claim 4, wherein the structural  
gene is *E. coli lacZ*.
6. The inhibitor of Claim 1, further comprising a  
transcriptional signal and a translational signal  
25 suitable for expression of the gene portion in a  
host cell.

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7. The inhibitor of Claim 6, wherein the transcriptional signal and the translational signal are those of the *ced-3* gene.
- 5 8. An inhibitor of the activity of the *ced-3* gene, comprising RNA encoded by the sense strand of a nucleotide sequence of Figure 3 (SEQ ID NO:1), the nucleotide sequence selected from the group consisting of:
  - 10 a) nucleotides 1 to approximately 5850;
  - b) nucleotides 1 to approximately 3020; and
  - c) an inhibitory subportion of (a) and (b).
- 15 9. An inhibitor of the activity of the *ced-3* gene, comprising protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
  - a) amino acids 1 to approximately 372;
  - b) amino acids 1 to approximately 149; and
  - c) an inhibitory subportion of (a) and (b).
- 20 10. An inhibitor of the activity of the *ced-3* gene, comprising a non-peptide mimetic of the inhibitor of Claim 9.
11. The inhibitor of Claim 1, consisting essentially of a construct selected from BGAFQ and PBA.
- 25 12. The inhibitor of Claim 1, comprising the encoded product of a construct selected from BGAFQ and PBA.

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13. The inhibitor of Claim 1, comprising a non-peptide mimetic of the protein encoded by a construct selected from BGAFQ and PBA.
- 5 14. An inhibitor of the activity of the *ced-3* gene, comprising protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
- a) amino acids 1 to 298;
  - b) amino acids 1 to 111; and
  - 10 c) an inhibitory subportion of (a) and (b).
- 15 15. An inhibitor of the activity of the *ced-3* gene, comprising a portion of the ICE gene which encodes the protein of Claim 14, or an inhibitory subportion of said gene.
- 15 16. An inhibitor of the activity of the *ced-3* gene, comprising RNA encoded by the gene portion of Claim 15.
17. An inhibitor of the activity of the *ced-3* gene, comprising a non-peptide mimetic of the protein of Claim 14.
- 20 18. An inhibitor of the activity of the *ced-3* gene, comprising a portion of the protein product of a gene which is structurally related to the *ced-3* gene, said protein portion corresponding to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
- 25 a) amino acids 1 to approximately 372;

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- b) amino acids 1 to approximately 149; and
  - c) an inhibitory subportion of (a) and (b).
19. An inhibitor of the activity of the *ced-3* gene,  
comprising a portion of a gene which is  
5 structurally related to the *ced-3* gene, said gene  
portion encoding the protein of Claim 18 or an  
inhibitory subsection of said gene portion.
20. An inhibitor of the activity of the *ced-3* gene,  
comprising RNA encoded by the gene portion of Claim  
10 19.
21. An inhibitor of the activity of the *ced-3* gene,  
comprising a non-peptide mimetic of the protein  
portion of Claim 18.
22. An inhibitor of the activity of the ICE gene  
15 comprising a portion of said gene which encodes an  
amino sequence of ICE shown in Figures 6A-B (SEQ ID  
NO:4), selected from the group consisting of:  
a) amino acids 1 to approximately 298;  
b) amino acids 1 to approximately 111; and  
20 c) an inhibitory subportion of (a) and (b).
23. The inhibitor of Claim 22, further comprising a  
heterologous structural gene fused 3' of the gene  
portion.
24. The inhibitor of Claim 22, further comprising a  
25 transcriptional signal and a translational signal  
suitable for expression of the gene portion in a  
host cell.

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25. An inhibitor of the activity of the ICE gene,  
comprising RNA encoded by the gene portion of Claim  
22.
- 5 26. An inhibitor of the activity of the ICE gene,  
comprising an amino acid sequence of ICE shown in  
Figures 6A-B (SEQ ID NO:4), selected from the group  
consisting of:  
a) amino acids 1 to approximately 298;  
b) amino acids 1 to approximately 111; and  
10 c) an inhibitory subportion of (a) and (b).
27. An inhibitor of the activity of the ICE gene,  
comprising a non-peptide mimetic of the protein of  
Claim 26.
- 15 28. An inhibitor of the activity of the ICE gene,  
comprising a portion of the *ced-3* gene.
29. The inhibitor of Claim 28, wherein said gene  
portion is a nucleotide sequence of Figure 3 (SEQ  
ID NO:1), selected from the group consisting of:  
a) nucleotides 1 to approximately 5850;  
20 b) nucleotides 1 to approximately 3020; and  
c) an inhibitory subportion of (a) and (b).
- 25 30. The inhibitor of Claim 28, wherein the gene portion  
encodes an amino acid sequence of the Ced-3 protein  
shown in Figures 6A-B (SEQ ID NO:2), selected from  
the group consisting of:  
a) amino acids 1 to approximately 372;  
b) amino acids 1 to approximately 149; and  
c) an inhibitory subportion of (a) and (b).

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31. The inhibitor of Claim 28, further comprising a heterologous structural gene fused 3' of the gene portion.
- 5 32. The inhibitor of Claim 28, further comprising a transcriptional signal and a translational signal suitable for expression of the gene portion in a host cell.
- 10 33. An inhibitor of the activity of the ICE gene, comprising RNA encoded by the sense strand of a portion of the *ced-3* gene, said gene portion which is a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
- a) nucleotides 1 to approximately 5850;
  - b) nucleotides 1 to approximately 3020; and
  - 15 c) an inhibitory subportion of (a) and (b).
34. An inhibitor of the activity of the ICE gene, comprising protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
- 20 a) amino acids 1 to approximately 372;
  - b) amino acids 1 to approximately 149; and
  - c) an inhibitory subportion of (a) and (b).
35. An inhibitor of the activity of the ICE gene, comprising a non-peptide mimetic of the protein of
- 25 Claim 34.
36. The inhibitor of Claim 28, consisting essentially of a construct selected from BGAFQ and PBA.

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37. An inhibitor of the activity of the ICE gene, comprising the encoded product of a construct selected from BGAFQ and PBA.
- 5 38. An inhibitor of the activity of the ICE gene comprising a portion of the protein product of a gene which is structurally related to said ICE gene, said protein portion corresponding to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group  
10 consisting of:  
a) amino acids 1 to approximately 372;  
b) amino acids 1 to approximately 149; and  
c) an inhibitory subportion of (a) and (b).
- 15 39. An inhibitor of the activity of the ICE gene, comprising a portion of a gene which is structurally related to the ICE gene, said gene portion encoding the protein of Claim 38, or an inhibitory subsection of said gene portion.
- 20 40. An inhibitor of the activity of the ICE gene, comprising RNA encoded by the gene portion of Claim 39.
41. An inhibitor of the activity of the ICE gene, comprising a non-peptide mimetic of the protein of Claim 38.
- 25 42. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising DNA selected from the group consisting of:

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- a) a portion of the nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
- 1) nucleotides 1 to approximately 5850;
  - 5 2) nucleotides 1 to approximately 3020; and
  - 3) an inhibitory subportion of (a.1) and (a.2);
- b) DNA encoding an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
- 10 1) amino acids 1 to approximately 372;
  - 2) amino acids 1 to approximately 149; and
  - 3) an inhibitory subportion of (b.1) and (b.2);
- 15 c) a portion of the ICE gene which encodes an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
- 20 1) amino acids 1 to approximately 298;
  - 2) amino acids 1 to approximately 111; and
  - 3) an inhibitory subportion of (c.1) and c.2);
- d) a portion of said *ced-3*/ICE gene which encodes an amino acid sequence corresponding to a portion of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), said Ced-3 portion selected from the group consisting of:
- 25 1) amino acids 1 to approximately 372;
  - 2) amino acids 1 to approximately 149; and
  - 30 3) an inhibitory subportion of (d.1) and (d.2); and



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- 5 e) a portion of a *ced-3*/ICE gene other than said *ced-3*/ICE gene which encodes an amino acid sequence corresponding to a portion of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), said Ced-3 portion selected from the group consisting of:
- 1) amino acids 1 to approximately 372;
  - 2) amino acids 1 to approximately 149; and
  - 10 3) an inhibitory subportion of (e.1) and (e.2).
43. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising RNA encoded by the DNA of Claim 42.
- 15 44. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising protein encoded by the DNA of Claim 42.
- 20 45. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising a non-peptide mimetic of the protein of Claim 44.
- 25 46. A drug for reducing cell deaths, comprising an inhibitor of the activity of the *ced-3* gene, selected from the group consisting of:
- a) a portion of the *ced-3* gene;
  - b) a product encoded by a portion of the *ced-3* gene;
  - c) a non-peptide mimetic of an inhibitory portion of the Ced-3 protein;
  - d) a portion of the ICE gene;

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- e) a product encoded by a portion of the ICE gene;
- f) a non-peptide mimetic of an inhibitory portion of the ICE protein;
- 5 g) a portion of a gene which is structurally related to the *ced-3* gene;
- h) a product encoded by the gene portion of (g); and
- 10 i) a non-peptide mimetic of the protein encoded by the gene portion of (g).

47. The drug of Claim 46, wherein the inhibitor is selected from the group consisting of:

- 15 a) DNA having a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
  - 1) nucleotides 1 to approximately 5850;
  - 2) nucleotides 1 to approximately 3020; and
  - 3) an inhibitory portion of (a.1) and (a.2);
- 20 b) DNA encoding an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from:
  - 1) amino acids 1 to approximately 372;
  - 2) amino acids 1 to approximately 149; and
  - 3) an inhibitory portion of (b.1) and (b.2);
- 25 c) RNA encoded by DNA of (a);
- d) RNA encoded by DNA of (b);
- e) protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
  - 30 1) amino acids 1 to approximately 372;
  - 2) amino acids 1 to approximately 149; and

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- 3) inhibitory portion of (e.1) and (e.2);  
and  
f) a non-peptide mimetic of the protein of e).
48. The drug of Claim 46, wherein the inhibitor is  
5 selected from the group consisting of:  
a) DNA encoding an amino acid sequence of ICE  
shown in Figures 6A-B (SEQ ID NO:4), selected  
from the group consisting of:  
1) amino acids 1 to approximately 298;  
10 2) amino acids 1 to approximately 111; and  
3) an inhibitory portion of (a.1) and (a.2);  
b) RNA encoded by DNA of a);  
c) protein having an amino acid sequence of ICE  
shown in Figures 6A-B (SEQ ID NO:4), selected  
15 from the group consisting of:  
1) amino acids 1 to approximately 298;  
2) amino acids 1 to approximately 111; and  
3) an inhibitory portion of (c.1) and (c.2);  
and  
20 d) a non-peptide mimetic of the protein of c).
49. The drug of Claim 46, wherein the inhibitor is  
selected from the group consisting of:  
a) protein encoded by a portion of a gene which  
is structurally related to the *ced-3* gene,  
25 said protein portion corresponding to an amino  
acid sequence of the Ced-3 protein shown in  
Figures 6A-B (SEQ ID NO:2) selected from the  
group consisting of:  
1) amino acids 1 to approximately 372;  
30 2) amino acids 1 to approximately 149; and  
3) an inhibitory portion of (a.1) and (a.2);

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- b) DNA encoding the protein of (a) or inhibitory subportion thereof;
  - c) RNA encoding the protein of (a) or inhibitory subportion thereof; and
  - 5 d) a non-peptide mimetic of the protein of (a).
50. An inhibitor as defined in Claim 46, for use in therapy e.g. for treating a condition characterized by cell deaths.
51. A method for treating a condition characterized by  
10 cell deaths, comprising administering the drug of Claim 46.
52. A drug for reducing cell deaths, comprising an inhibitor of the activity of the ICE gene, selected from the group consisting of:
- 15 a) a portion of the ICE gene;
  - b) a product encoded by a portion of the ICE gene;
  - c) a non-peptide mimetic of an inhibitory portion of the ICE protein;
  - 20 d) a portion of the *ced-3* gene;
  - e) a product encoded by a portion of the *ced-3* gene;
  - f) a non-peptide mimetic of an inhibitory portion of the Ced-3 protein;
  - 25 g) a portion of a gene which is structurally related to the *ced-3* gene and the ICE gene;
  - h) a product encoded by the gene portion of (e); and
  - 30 i) a non-peptide mimetic of the protein encoded by (g).

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53. A drug for reducing cell deaths, comprising an inhibitor of the activity of a gene which is structurally related to the *ced-3* gene and ICE gene, selected from the group consisting of:
- 5 a) a portion of said related gene;  
b) a product encoded by the gene portion of (e);  
c) a non-peptide mimetic of the protein product encoded by (a);  
d) a portion of the ICE gene;  
10 e) a product encoded by the gene portion of (d);  
f) a non-peptide mimetic of a protein product encoded by (d);  
g) a portion of the *ced-3* gene;  
h) a product encoded by the gene portion of (g);  
15 and  
i) a non-peptide mimetic of the protein product encoded by (g).
54. A drug for inhibiting the activity of a gene selected from the group consisting of *ced-3* and a  
20 gene which belongs to the *ced-3*/ICE gene family, comprising an inhibitor of interleukin-1 $\beta$  convertase.
55. The drug of Claim 54 which reduces cell deaths.
56. The drug of Claim 54 which is a peptide aldehyde  
25 containing the amino acid sequence Tyr-Val-X-Asp, wherein X is selected from Ala, His, Gln, Lys, Phe, Cha, and Asp.
57. The drug of Claim 54 which is Ac-Tyr-Val-Ala-Asp-CHO, also referred to as inhibitor B.

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58. The drug of Claim 54 which is the cowpox virus CrmA protein.
59. An anti-inflammatory drug, comprising an inhibitor of the activity of the ICE gene, or inhibitory portion thereof, selected from the group consisting of:
- a) a portion of the ICE gene;
  - b) a product encoded by a portion of the ICE gene;
  - 10. c) a portion of the *ced-3* gene;
  - d) a product encoded by a portion of the *ced-3* gene;
  - e) a portion of a gene which is structurally related to the *ced-3* gene and ICE gene; and
  - 15 f) a product encoded by a portion of a gene which is structurally related to the *ced-3* gene and the ICE gene.
60. The anti-inflammatory drug of Claim 59, wherein the inhibitor is selected from the group consisting of:
- 20 a) DNA encoding an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
    - 1) amino acids 1 to approximately 298;
    - 2) amino acids 1 to approximately 111; and
    - 25 3) an inhibitory portion of (a.1) and (a.2);
  - b) RNA encoded by DNA of (a) or an inhibitory subportion thereof;
  - c) protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
    - 30 1) amino acids 1 to approximately 298;

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- 2) amino acids 1 to approximately 111; and
- 3) an inhibitory portion of (c.1) and (c.2);
- d) a non-peptide mimetic of the protein of (c).

61. The anti-inflammatory drug of Claim 59, wherein the  
5 inhibitor is selected from the group consisting of:
- a) DNA having a nucleotide sequence of Figure 3  
(SEQ ID NO:1), selected from the group  
consisting of:
    - 1) nucleotides 1 to approximately 5850;
    - 10 2) nucleotides 1 to approximately 3020; and
    - 3) an inhibitory portion of (a.1) and (a.2);
  - b) DNA encoding an amino acid sequence of the  
Ced-3 protein shown in Figures 6A-B (SEQ ID  
NO:2), selected from the group consisting of:
    - 15 1) amino acids 1 to approximately 372;
    - 2) amino acids 1 to approximately 149; and
    - 3) an inhibitory portion of (b.1) and (b.2);
  - c) RNA encoded by DNA of (a);
  - d) RNA encoded by DNA of (b);
  - 20 e) protein having an amino acid sequence of the  
Ced-3 protein shown in Figures 6A-B (SEQ ID  
NO:2), selected from the group consisting of:
    - 1) amino acids 1 to approximately 372;
    - 2) amino acids 1 to approximately 149; and
    - 25 3) an inhibitory portion of (e.1) and (e.2);  
and
  - f) a non-peptide mimetic of the protein of (e).

62. The anti-inflammatory drug of Claim 59, wherein the  
inhibitor is selected from the group consisting of:

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- 5 a) protein encoded by a portion of a gene which is structurally related to the *ced-3* and ICE genes, said protein portion corresponding to an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
- 1) amino acids 1 to approximately 298;
  - 2) amino acids 1 to approximately 111; and
  - 3) an inhibitory portion of (a.1) and (a.2);
- 10 b) DNA encoding the protein of (a);
- c) RNA encoding the protein of (a); and
- d) a non-peptide mimetic of the protein of (a).
63. A method for treating inflammation, comprising administering the drug of Claim 59.
- 15 64. An inhibitor or inhibitory portion as defined in Claim 59, for use in therapy e.g. for treating inflammation.
65. A method for altering the occurrence of cell death, comprising altering the activity of a cell death gene which is structurally related to *ced-3*.
- 20 66. The method of Claim 65, wherein the structurally related gene is ICE.
67. A drug for increasing cell deaths, comprising a molecule, of active portion thereof, selected from:
- 25 a) DNA comprising a gene which belongs to the *ced-3*/ICE gene family;
- b) RNA encoded by the DNA of (a);
- c) protein encoded by the DNA of (a);



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- d) an agent which is structurally similar to and mimics the activity of the protein of (c);
- e) an agonist of the activity of a gene which belongs to the *ced-3*/ICE gene family;
- 5 f) DNA comprising a constitutively activated mutated form of a gene which belongs to the *ced-3*/ICE gene family;
- g) RNA encoded by the DNA of (e);
- h) protein encoded by the DNA of (e);
- 10 i) an agent which is structurally similar to and mimics the activity of a protein encoded by the DNA of (e); and
- j) an agonist of the activity of a constitutively activated mutated form of a gene which belongs to the *ced-3*/ICE gene family.
- 15

68. The drug of Claim 67, wherein the gene which belongs to the *ced-3*/ICE gene family is ICE.

69. The drug of Claim 67(f), wherein the constitutively activated mutated form of the gene which belongs to the *ced-3*/ICE gene family encodes a carboxyl-terminal portion of a protein product of the wild-type gene, said carboxyl-terminal portion having a deletion of an amino-terminal portion which corresponds to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2),
- 20
- 25
- selected from the group consisting of:
- a) amino acids 1 to approximately 372;
  - b) amino acids 1 to approximately 149; and
  - c) an inhibitory subportion of (a) and (b).

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70. The drug of Claim 69, wherein the protein product of the wild-type gene has sequences corresponding to the autocleavage sites of ICE and the protein product of the wild-type gene is selected from the group consisting of:
- a) the uncleaved form of the protein product; and
  - b) the subunits corresponding to the active subunits of ICE.
71. A method for reducing the proliferative capacity or size of a population of cells, comprising contacting the cells with the drug of Claim 67 under conditions suitable for activity of the drug.
72. The method of Claim 71, wherein the population of cells is selected from the group consisting of:
- a) cancerous cells;
  - b) cells which produce autoreactive antibodies;
  - c) infected cells;
  - d) hair follicle cells;
  - e) cells which are critical to the life of a parasite;
  - f) cells which are critical to the life of a pest; and
  - g) cells which are critical to the life of a recombinant organism.
73. A molecule or active portions thereof as defined in Claim 67, for use in therapy e.g. for reducing the proliferative capacity or size of population of cells, selected for example from the group consisting of:
- a) cancerous cells;

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- b) cells which produce autoreactive antibodies;
  - c) infected cells;
  - d) hair follicle cells;
  - e) cells which are critical to the life of a  
5 parasite;
  - f) cells which are critical to the life of a  
pest; and
  - g) cells which are critical to the life of a  
recombinant organism.
- 10 74. A drug for decreasing cell deaths comprising a  
molecule selected from the group consisting of:
- a) single stranded nucleic acid having all or a  
portion of the antisense sequence of a gene  
which is structurally related to *ced-3*, said  
15 nucleic acid which is complementary to the  
mRNA of the gene;
  - b) DNA which directs the expression of (a);
  - c) a mutated form of a gene which is structurally  
related to *ced-3*, does not cause cell death  
20 and antagonizes the activity of the wild-type  
gene; and
  - d) an antagonist of the activity of a gene which  
is structurally related to *ced-3*.
- 25 75. The drug of Claim 74, wherein the structurally  
related gene is ICE.
76. A molecule as defined in Claim 74, for use in  
therapy e.g. for treating, in a human or other  
animal, a condition characterized by cell deaths,  
and wherein for example the condition is selected  
30 from the group consisting of:

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- a) myocardial infarction;
  - b) stroke;
  - c) degenerative disease;
  - d) traumatic brain injury;
  - 5 e) hypoxia;
  - f) pathogenic infection; and
  - g) hair loss.
77. A method for treating, in a human or other animal, a condition characterized by cell deaths,
- 10 comprising administering the drug of Claim 74 to the human or other animal under conditions suitable for activity of the drug.
78. The method of Claim 77, wherein the condition is selected from the group consisting of:
- 15 a) myocardial infarction;
- b) stroke;
- c) degenerative disease;
- d) traumatic brain injury;
- e) hypoxia;
- 20 f) pathogenic infection; and
- g) hair loss.
79. A constitutively activated cell death protein comprising an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2),
- 25 selected from the group consisting of:
- a) the amino acids from approximately 150 to 503;
- b) the amino acids from approximately 373 to 503;
- c) the amino acids from approximately 150 to 372;
- d) (b) and (c) together;
- 30 e) an active subportion of (a), (b), and (c); and

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f) combinations of these.

80. The constitutively activated cell death protein of Claim 79, further comprising a subportion of the region of Ced-3 from amino acids 1 to 149, as shown in Figures 6A-B (SEQ ID NO:2), said subportion which enhances and does not inhibit the activity of the protein.
81. Isolated nucleic acid encoding the protein of Claim 79.
82. A constitutively activated cell death protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
- a) the amino acids from approximately 111 to 404;
  - b) the amino acids from approximately 298 to 404;
  - c) the amino acids from approximately 111 to 297;
  - d) (b) and (c) together;
  - e) an active subportion of (a), (b), and (c); and
  - f) combinations of these.
83. Isolated nucleic acid encoding the protein of Claim 82.
84. A drug for increasing cell deaths, comprising a molecule selected from the protein of Claim 79 or a nucleic acid encoding said protein.
85. A drug for increasing cell deaths, comprising a molecule selected from the protein of Claim 79 or a nucleic acid encoding said protein.

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86. Isolated protein having cell death activity and the amino acid sequence of the NEDD-2 protein shown in Figure 7 (SEQ ID NO:13), or an active portion thereof.
- 5 87. Isolated nucleic acid encoding the protein of Claim 86.
88. An isolated substrate-specific protease having the amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2).
- 10 89. An isolated substrate-specific protease, consisting essentially of a protein product of a gene which is structurally related to the *ced-3* and ICE genes.
90. The protease of Claim 89 which cleaves after aspartate residues.
- 15 91. The protease of Claim 89 which is a cysteine protease.
92. Isolated ICE having an alteration which reduces the activity of the enzyme, the alteration selected from the group consisting of:
- 20 a) L to F at amino acid 26;  
b) G to R at amino acid 65;  
c) G to S at amino acid 287;  
d) E to termination at amino acid 324;  
e) W to termination at amino acid 340;
- 25 f) A to V at amino acid 361;  
g) E to K at amino acid 390; and  
h) T to F at amino acid 393.

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93. Isolated DNA which is a mutated ICE gene encoding the altered enzyme of Claim 92.
94. Isolated RNA encoded by the DNA of Claim 93.
- 5 95. An isolated gene belonging to the *ced-3*/ICE family of structurally related genes which has a mutation which reduces the activity of the gene, said mutation resulting in an amino acid alteration corresponding to an amino acid alteration of the Ced-3 protein which inactivates the Ced-3 protein.
- 10 96. A product of the gene of Claim 95 selected from RNA and protein.
97. Isolated protein which is the NEDD-2 protein having an alteration which inactivates the protein, said alteration selected from the group consisting of:
- 15 a) A to V at amino acid 117;  
b) E to K at amino acid 483; and  
c) S to F at amino acid 486.
98. Isolated nucleic acid encoding the protein of Claim 97.
- 20 99. Isolated protein which is structurally similar to Ced-3 and has an alteration at a conserved amino acid corresponding to an amino acid of the Ced-3 protein selected from the group consisting of:
- 25 a) Ser 183;  
b) Met 234;  
c) Arg 242;  
d) Leu 246;

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e) Ile 247;  
f) Ile 248;  
g) Asn 250;  
h) Phe 253;  
5 i) Arg 259;  
j) Gly 261;  
k) Asp 265;  
l) Gly 277;  
m) Tyr 278;  
10 n) Val 280;  
o) Lys 283;  
p) Asn 285;  
q) Leu 286;  
r) Thr 287;  
15 s) Met 291;  
t) Phe 298;  
u) His 304;  
v) Asp 306;  
w) Ser 307;  
20 x) Leu 310;  
y) Val 311;  
z) Ser 314;  
aa) His 315;  
bb) Gly 316;  
25 cc) Ile 321;  
dd) Gly 323;  
ee) Ile 334;  
ff) Asn 339;  
gg) Pro 344;  
30 hh) Leu 346;  
ii) Lys 349;  
jj) Pro 350;  
kk) Lys 351;



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11) Gln 356;  
mm) Ala 357;  
nn) Cys 358;  
oo) Arg 359;  
5 pp) Gly 360;  
qq) Asp 371;  
rr) Asp 414;  
ss) Arg 429;  
tt) Gly 434;  
10 uu) Ser 435;  
vv) Ile 438;  
ww) Ala 449;  
xx) Val 452;  
yy) Leu 488;  
15 aa) Tyr 493; and  
aaa) Pro 496.

100. Isolated nucleic acid encoding the protein of Claim 99.

101. A method for identifying a portion of the *ced-3*  
20 gene which inhibits the activity of the *ced-3*  
gene, comprising the steps of:  
a) injecting wild-type nematodes with a portion  
of the *ced-3* gene under conditions suitable  
for expression of said gene portion; and  
25 b) detecting a decrease in programmed cell  
deaths,  
whereby a decrease in programmed cell deaths is  
indicative of a portion of the *ced-3* gene which  
inhibits the activity of said gene.

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102. Isolated DNA comprising the inhibitory portion of the *ced-3* gene identified by the method of Claim 101.

5 103. A method for identifying a portion of a gene which is structurally related to the *ced-3* gene which inhibits the activity of the *ced-3* gene, comprising the steps of:

10 a) injecting wild-type nematodes with a portion of the structurally related gene under conditions suitable for expression of said gene portion; and

b) detecting a decrease in programmed cell deaths,

15 whereby a decrease in programmed cell deaths is indicative of a portion of the structurally related gene which inhibits the activity of the *ced-3* gene.

104. Isolated DNA comprising the inhibitory portion of the structurally related gene identified by the method of Claim 103.

20 105. The DNA of Claim 104, wherein the structurally related gene is the ICE gene.

106. A method for identifying a portion of ICE which inhibits the activity of said enzyme, comprising the steps of:

25 a) combining a portion of ICE with ICE and a substrate of ICE under conditions suitable for cleavage of the substrate by ICE; and  
b) detecting a decrease in cleavage of the substrate,

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whereby a decrease in cleavage of the substrate is indicative of a portion of ICE which inhibits the activity of said enzyme.

107. An isolated inhibitory portion of the ICE protein  
5 identified by the method of Claim 106.

108. Isolated nucleic acid encoding the inhibitory  
portion of Claim 107.

109. A method for identifying a portion of the protein  
product of a gene which is structurally related to  
10 the *ced-3* and ICE genes, and inhibits the activity  
of ICE, comprising the steps of:

a) combining a portion of the protein product of  
a gene which is structurally related to the  
*ced-3* and ICE genes with ICE and a substrate  
15 of ICE under conditions suitable for cleavage  
of the substrate by ICE; and

b) detecting a decrease in cleavage of the  
substrate,  
whereby a decrease in cleavage of the substrate is  
20 indicative of a portion of the protein product of a  
gene which is structurally related to the *ced-3* and  
ICE genes and inhibits the activity of ICE.

110. An isolated inhibitory portion identified by the  
method of Claim 109.

25 111. Isolated nucleic acid encoding the inhibitory  
portion of Claim 110.

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112. A method for identifying a gene which is structurally related to the *ced-3* gene and the ICE gene, comprising detecting a gene with:
- 5 a) a probe derived from the *ced-3* gene or a product encoded by the *ced-3* gene; and
  - b) a probe derived from the ICE gene or a product encoded by the ICE gene.
113. An isolated gene identified by the method of Claim 112.
- 10 114. A method for identifying a gene which belongs to the *ced-3*/ICE family of structurally related genes, comprising detecting a gene with a probe selected from the group consisting of:
- 15 a) a probe derived from a gene which is structurally related to the *ced-3* gene and the ICE gene; and
  - b) a probe derived from the consensus sequence of a conserved region in genes belonging to the
- 20 *ced-3*/ICE gene family.
115. An isolated gene identified by the method of Claim 114 which has an activity selected from cell death activity and protease activity.
116. Isolated DNA selected from the group consisting of:
- 25 a) a region of a gene belonging to the *ced-3*/ICE family of structurally related genes which is conserved among two or more family members; and

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b) the consensus sequence of a conserved region  
in genes belonging to the *ced-3*/ICE gene  
family,  
or encoded product thereof.

5 117. A method for identifying a gene which interacts  
with a *ced-3*/ICE gene belonging to said family,  
comprising identifying a mutation which enhances or  
suppresses the activity of a *ced-3*/ICE gene in a  
nematode, wherein the enhancing or suppressing  
10 mutation is indicative of a gene which interacts  
with the *ced-3*/ICE gene.

118. The method of Claim 117, wherein the *ced-3*/ICE gene  
is selected from the group consisting of:  
a) a wild-type *ced-3* gene;  
15 b) a mutated *ced-3* gene, the nematode being a  
mutant nematode;  
c) a transgene which is a wild-type form of said  
*ced-3*/ICE gene, the nematode being a  
transgenic nematode having an inactivated  
20 endogenous *ced-3* gene; and  
d) a transgene which is a mutated form of said  
*ced-3*/ICE gene, the nematode being a  
transgenic nematode having an inactivated  
endogenous *ced-3* gene.

25 119. An isolated gene identified by the method of Claim  
117, or an encoded product thereof.

-103-

120. A bioassay for identifying an agent which affects the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising the steps of:
- 5 a) introducing an agent into a transgenic nematode which expresses a *ced-3*/ICE gene; and
  - b) detecting an alteration in the occurrence of cell deaths in the transgenic nematode, wherein an alteration indicates that the agent
  - 10 affects the activity of the *ced-3*/ICE gene.
121. The method of Claim 120, wherein the *ced-3*/ICE gene is selected from a wild-type gene and a mutated gene.
122. An agent identified by the method of Claim 120.
- 15 123. A diagnostic probe for a disease characterized by cell deaths, comprising a molecule selected from the group consisting of:
- a) all or a portion of the *ced-3* gene (SEQ ID NO:1) which is specific to said *ced-3* gene;
  - 20 b) RNA encoded by the *ced-3* gene;
  - c) degenerate oligonucleotides derived from the amino acid sequence of the Ced-3 protein (SEQ ID NO:2);
  - d) an antibody directed against the Ced-3
  - 25 protein;
  - e) all or a portion of the ICE gene (SEQ ID NO:3) which is specific to said ICE gene;
  - f) RNA encoded by the ICE gene;
  - 30 g) degenerate oligonucleotides derived from the amino acid sequence of ICE (SEQ ID NO:4);

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- h) an antibody directed against ICE;
- i) a gene which is structurally related to the *ced-3* gene, or portion thereof specific to said structurally related gene;
- 5 j) RNA encoded by the structurally related gene;
- k) degenerate oligonucleotides derived from the amino acid sequence of the protein product of a gene which is structurally related to *ced-3*; and
- 10 d) an antibody directed against the protein product of a gene which is structurally related to *ced-3*.

124. A method for diagnosis of a disease characterized by cell deaths, comprising detecting an abnormality  
15 in the sequence of a gene which is structurally related to *ced-3*.

125. The method of Claim 124, wherein the structurally related gene is ICE.

126. A method for diagnosis of a disease characterized  
20 by cell deaths, comprising detecting an abnormality in the activity of a gene which is structurally related to *ced-3*.

127. The method of Claim 126, wherein the structurally related gene is ICE.

25 128. A diagnostic probe for an inflammatory disease, comprising a molecule selected from the group consisting of:

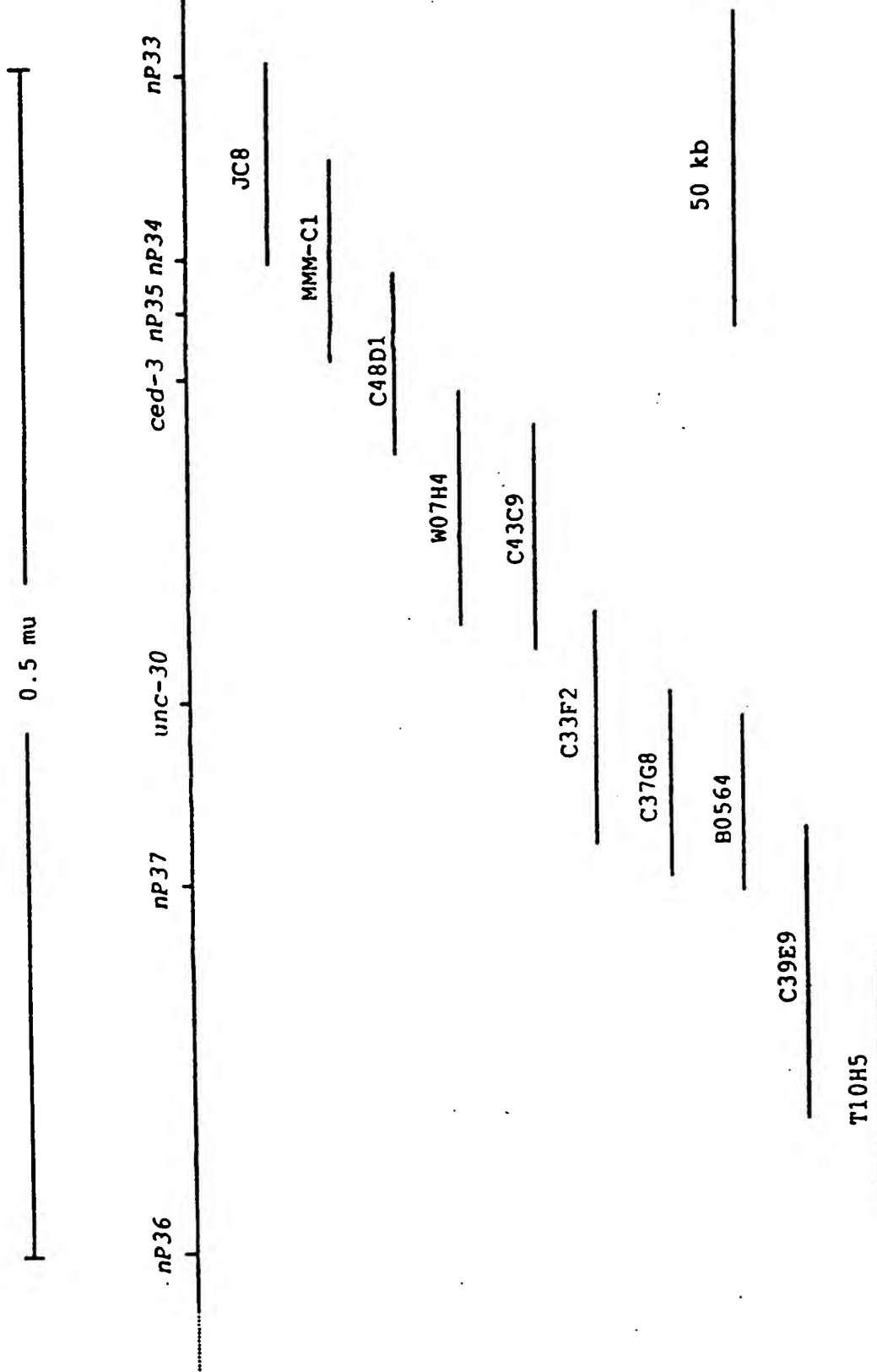
-105-

- a) all or a portion of the *ced-3* gene shown in Figure 3 (SEQ ID NO:1) which is specific to the *ced-3* gene;
  - b) RNA encoded by (a);
  - 5 c) degenerate oligonucleotides derived from the amino acid sequence of the Ced-3 protein as shown in Figures 6A-B (SEQ ID NO:2);
  - d) an antibody directed against the Ced-3 protein;
  - 10 e) a gene which is structurally related to the *ced-3* and ICE genes, or portion thereof which is specific for said related gene;
  - f) RNA encoded by (a);
  - 15 g) degenerate oligonucleotides derived from the amino acid sequence of the protein encoded by (e); and
  - h) an antibody directed against the protein encoded by (e).
129. A method for diagnosis of an inflammatory disease,  
20 comprising detecting an abnormality in the sequence of a gene which is a member of the *ced-3*/ICE gene family.
130. The method of Claim 129, wherein the gene is *ced-3*.
131. A method for diagnosis of an inflammatory disease,  
25 comprising detecting an abnormality in the activity of a gene which belongs to the *ced-3*/ICE gene family, or an encoded product thereof.
132. The method of Claim 131, wherein the gene is *ced-3*.



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FIGURE 1

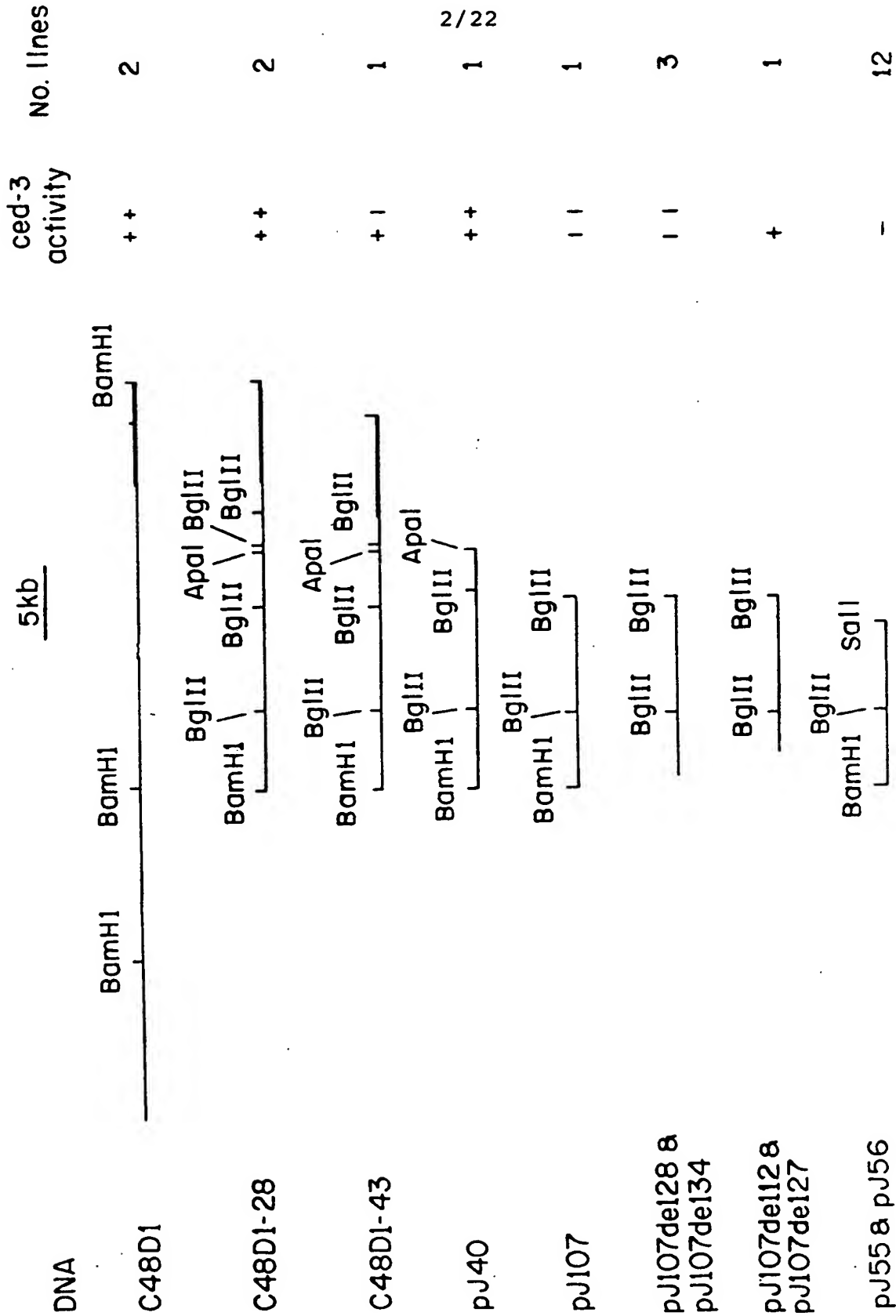


FIG. 2

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*ced-3* Genomic Sequence

```

1  AGATCTGAAATAAGGTGATAAATTAATAAATTAAGTGTATTTCTGAGGAAATTTGACTGT 60
   +-----+-----+-----+-----+-----+
61  TTTAGCACAAATTAATCTTGTTTCAGAAAAAAGTCCAGTTTTCTAGATTTTCCGTCTTA 120
   +-----+-----+-----+-----+-----+
121 TTGTCGAATTAATATCCCTATTATCACTTTTTTCATGCTCATCCTCGAGCGGCACGTCCTC 180
   +-----+-----+-----+-----+-----+
181 AAAGAATTGTGAGAGCAAACGCGCTCCCATTTGACCTCCACACTCAGCCGCCAAAAACAAC 240
   +-----+-----+-----+-----+-----+
241 GTTCGAACATTTCGTGTGTTGTGCTCCTTTCCGTTATCTTGCAAGTCATCTTTGTGCTTT 300
   +-----+-----+-----+-----+-----+
301 TTTTCTTTGTTCTTTTGTGTAACGTGTTGCTAAGCAATTATTACATCAATTGAAGAAAA 360
   +-----+-----+-----+-----+-----+
361 GGCTCGCGGATTTATGTTGCCAGAAAGATTCTGAGATTCTCGAAGTCGATTTTATAATA 420
   +-----+-----+-----+-----+-----+
421 TTTAACCTTGGTTTTTGCATTGTTTCGTTTAAAAAAACCAGTGTATGTGAAAAACGAT 480
   +-----+-----+-----+-----+-----+
481 TAGTTTACTAATAAACTACTTTTAAACCTTTACCTTTACCTCACCGCTCCGTGTTTCATG 540
   +-----+-----+-----+-----+-----+
541 GCTCATAGATTTTCGATACTCAAATCCAAAAATAAATTTACGAGGGCAATTAATGTGAAA 600
   +-----+-----+-----+-----+-----+
601 CAAAAACAATCCTAAGATTTCCACATGTTTGACCTCTCGGCACCTTCTTCCTTAGCCCC 660
   +-----+-----+-----+-----+-----+
661 ACCACTCCATCACCTCTTTGGCGGTGTTCTTCGAAACCCACTTAGGAAAGCAGTGTGTAT 720
   +-----+-----+-----+-----+-----+
721 CTCATTTGGTATGCTCTTTTCGATTTTATAGCTCTTTGTGCAATTTCAATGCTTTAAAC 760
   +-----+-----+-----+-----+-----+
781 AATCCAAATCGCATTATATTTGTGCATGGAGGCAATGACGGGGTTGGAATCTTAGATGA 840
   +-----+-----+-----+-----+-----+
841 GATCAGGAGCTTTCAGGGTAAACGCCGGTTTATTTTGTACCATTTTCATCATTTTCCT 900
   +-----+-----+-----+-----+-----+
901 GTCGTCCTTGGTATCCTCAACTTGTCCCGGTTTTGTTTTCGGTACACTCTTCCGTGATGC 960
   +-----+-----+-----+-----+-----+
961 CACCTGTCTCCGTCTCAATTATCGTTTAGAAATGTGAACTGTCCAGATGGGTGACTCATA 1020
   +-----+-----+-----+-----+-----+
1021 TTGCTGCTGCTACAATCCACTTTCTTTTCTCATCGGCAGTCTTACGAGCCCATCATAAAC 1080
   +-----+-----+-----+-----+-----+
1081 TTTTTTTTCCGCGAAATTTGCAATAAACCGGCCAAAAACTTTCTCAAATTTGTACGCAA 1140
   +-----+-----+-----+-----+-----+
1141 TATATACAATCCATAAGAATATCTTCTCAATGTTTATGATTTCTTCGCAGCACTTTCTCT 1200
   +-----+-----+-----+-----+-----+
1201 TCGTGTGCTAACATCTTATTTTATAATATTTCCGCTAAAATTCGATTTTTGAGTATTA 1260
   +-----+-----+-----+-----+-----+
1261 ATTTATCGTAAAATTATCATAATAGCACCGAAAATACTAAAAATGGTAAAAGCTCCTTT 1320
   +-----+-----+-----+-----+-----+

                                     Repeat 1
                                     +-----+
1321 TAAATCGGCTCGACATTATCGTATTAAGGAATCACAAAATTCTGAGAATGCGTACTGCGC 1380
   +-----+-----+-----+-----+-----+

-----+-----+-----+-----+-----+
1381 AACATATTTGACGGCAAAATATCTCGTAGCGAAAACACAGTAATTCTTTAAATGACTAC 1440
   +-----+-----+-----+-----+-----+

                                     Repeat 1
                                     +-----+
1441 TGTAGCGCTTGTGTCGATTTACGGGCTCAATTTTTGAAAATAATTTTTTTTTTCGAATTT 1500
   +-----+-----+-----+-----+-----+

```

FIGURE 3(a)

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1501  
TGATAACCCGTAATCGTCACAACGCTACAGTAGTCATTAAAGGATTACTGTAGTTCTA  
-----+-----+-----+-----+-----+-----+-----+  
1560

1561  
GCTACGAGATATTTTGC GCGCCAATATGACTGTAATACGCATTCTCTGAATTTTGTGTT  
-----+-----+-----+-----+-----+-----+-----+  
1620  
TCCGTAATAATTT CACAAGATTTTGGCATTCCACTTTAAAGGCGCACAGGATTTATTCCA  
-----+-----+-----+-----+-----+-----+-----+  
1680  
ATGGGTCTCGGCACGCCAAAAGTTTGATAGACTTTTAAATCTCCTTGCATTTTAAATTC  
-----+-----+-----+-----+-----+-----+-----+  
1740  
AATTACTAAAATTTTCGTGAATTTTTCTGTTAAAAATTTTAAAAATCAGTTTTCTAATATT  
-----+-----+-----+-----+-----+-----+-----+  
1800  
TTCCAGGCTGACAAACAGAAACAAAAACACAACAAACATTTTAAAAATCAGTTTTCAAAAT  
-----+-----+-----+-----+-----+-----+-----+  
1860  
TAAAAATAACGATTTCTCATTGAAAATTGTGTTTATGTTTGC GAAAATAAAAGAGAACT  
-----+-----+-----+-----+-----+-----+-----+  
1920  
GATTCAAACCAATTTTAAACAAAAAAAAAACCCAAAATTGCGCAGAAATCAAGATAAAAAA  
-----+-----+-----+-----+-----+-----+-----+  
1980  
TTCAAGAGGGTCAAAATTTTCCGATTTTACTGACTTTCACCTTTTTTTTCGTAGTTCAGT  
-----+-----+-----+-----+-----+-----+-----+  
2040  
GCAGTTGTTGGAGTTTTTGACGAAAAC TAGGAAAAAATCGATAAAAAATTACTCAATTCG  
-----+-----+-----+-----+-----+-----+-----+  
2100  
AGCTGAATTTTGAGGACAATGTTTAAAAAAAACACTATTTTCCAATAATTTCACTCAT  
-----+-----+-----+-----+-----+-----+-----+  
2160

2161  
-----  
TTTCAGACTAAATCGAAAATCAAATCGTACTCTGACTACGGGTCAGTAGAGAGGTCAACC  
-----  
-----+-----+-----+-----+-----+-----+-----+  
2220

2221  
ATCAGCCGAAGATGATGCGTCAAGATAGAAGGAGCTTGCTAGAGAGGAACATTATGATGT  
-----+-----+-----+-----+-----+-----+-----+  
2280  
M M R Q D R R S L E R N I M M F  
1 10  
T(n1040)  
|

2281  
TCTCTAGTCATCTAAAAGTCGATGAAATTTCTCGAAGTTCTCATCGCAAAACAAGTGTGA  
-----+-----+-----+-----+-----+-----+-----+  
2340  
S S H L K V D E I L E V L I A K Q V L N  
20 30

2341  
|intron 1  
ATAGTGATAATGGAGATATGATTAATGTGAGTTTTTAATCGAATAATAATTTTAAAAAAA  
-----+-----+-----+-----+-----+-----+-----+  
2400  
S D N G D M I N  
40

2401  
AATTGATAATATAAAGAATATTTTTTGCAAGTCATGTGGAACGGTTCGCGAGAAGAGACGGG  
-----+-----+-----+-----+-----+-----+-----+  
2460  
S C G T V R E K R R E  
50  
A(n718)  
|

2461  
AGATCGTGAAAGCAGTGCAACGACGGGAGATGTGGCGTTCGACGCGTTTTATGATGCTC  
-----+-----+-----+-----+-----+-----+-----+  
2520  
I V K A V Q R R G D V A F D A F Y D A L  
60 70

2521  
|intron 2  
TTCGCTCTACGGGACACGAAGGACTTGCTGAAGTTCTTGAACCTCTCGCCAGATCGTAGG  
-----+-----+-----+-----+-----+-----+-----+  
2580  
R S T G H E G L A E V L E P L A R S  
80 90

**FIGURE 3(b)**

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```

2581 TTTTAAAGTTCGGCGCAAAAGCAAGGGTCTCACGGAAAAAAGAGGCGGATCGTAATTTT 2640
-----+-----+-----+-----+-----+-----+-----+-----+
2641 GCAACCCACCGGCACGGTTTTTTCCTCCGAAAATCGGAAATTATGCACTTTCCCAAATAT 2700
-----+-----+-----+-----+-----+-----+-----+-----+
2701 TTGAAGTGAATATATTTTATTTACTGAAAGCTCGAGTGATTATTTATTTTAACTA 2760
-----+-----+-----+-----+-----+-----+-----+-----+
2761 ATTTTCGTGGCGCAAAAGGCCATTTTGTAGATTTGCCGAAAATACTTGTACACACACAC 2820
-----+-----+-----+-----+-----+-----+-----+-----+

2821 ACACACATCTCCTTCAAATATCCCTTTTTCCAGTGTTGACTCGAATGCTGTGCAATTCTGA 2880
-----+-----+-----+-----+-----+-----+-----+-----+
                               V D S N A V E F E
                               100

2881 GTGTCCAATGTCACCGGCAAGCCATCGTCGGAGCCGCGCATTGAGCCCCGCCGGCTACAC 2940
-----+-----+-----+-----+-----+-----+-----+-----+
      C P M S P A S H R R S R A L S P A G Y T
      110                               120

2941 TTCACCGACCCGAGTTCACCGTGACAGCGTCTCTTCAGTGTCATTCATTCTTATCA 3000
-----+-----+-----+-----+-----+-----+-----+-----+
      S P T R V H R D S V S S V S S F T S Y Q
      130                               140

3001 GGATATCTACTCAAGAGCAAGATCTCGTTCTCGATCGCGTGCACTTCATTTCATCGGATCG 3060
-----+-----+-----+-----+-----+-----+-----+-----+
      D I Y S R A R S R S R S R A L H S S D R
      150                               160

3061 ACACAATTATTTCATCTCCTCCAGTCAACGCATTTCCAGCCAACCTTGTATGTTGATGCG 3120
-----+-----+-----+-----+-----+-----+-----+-----+
      H N Y S S P P V N A F P S Q P S
      170

Repeat 1
-----+-----+-----+-----+-----+-----+-----+-----+
3121 AACACTAAATTCTGAGAATGCGCATTACTCAACATATTTGACGCGCAAATATCTCGTAGC 3180
-----+-----+-----+-----+-----+-----+-----+-----+

3181 GAAAAATACAGTAACCCCTTTAAATGACTATTGTAGTGTGATTTACGGGCTCGATTTTCG 3240
-----+-----+-----+-----+-----+-----+-----+-----+

3241 ==> AAACGAATATATGCTCGAATTGTGACAACGAATTTTAATTTGTCAATTTTGTGTTTTCTT 3300
-----+-----+-----+-----+-----+-----+-----+-----+

3301 <-----+-----+-----+-----+-----+-----+-----+-----+
Repeat 1
      TTGATATTTTGTGATCAATTAATAAATTATTTCCGTAAACAGACACCAGCGCTACAGTACT 3360
-----+-----+-----+-----+-----+-----+-----+-----+

3361 CTTTTAAAGAGTTACAGTAGTTTTTCGCTTCAAGATATTTTGAAGAATTTTAAACATTT 3420
-----+-----+-----+-----+-----+-----+-----+-----+

3421 TGAATAAATCATCTAACATGTGCCAAAACGCTTTTTTCAAGTTTCGCAGATTTTGTGA 3480
-----+-----+-----+-----+-----+-----+-----+-----+

```

FIGURE 3(c)

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		7/22	
4321	TCACCGGATGCTCTTCTCTCGGATACAGTTCAAGTCGTAATCGCTCATTCAGCAAAGCTT	4380	
	T G C S S L G Y S S S R N R S F S K A S		
	190 200		
4381	CTGGACCAACTCAATACATATTTCCATGAAGAGGATATGAACCTTGTCGATGCACCAACCA	4440	
	G P T Q Y I F H E E D M N F V D A P T I		
	210 220		
4441	TAAGCCGTGTTTTTCGACGAGAAAAACCATGTACAGAACTTCTCGASTCCTCGTGGAATGT	4500	
	S R V F D E K T M Y R N F S S P R G M C		
	230 240		
4501	GCCTCATCATAAATAATGAACACTTTGAGCAGATGCCAACACGGAAATGGTACCAAGGCCG	4560	
	L I I N N E H F E Q M P T R N G T K A D		
	250 260		
4561	ACAAGGACAATCTTACCAATTTGTTTCAGATGCATGGGCTATACGGTTATTTGCAAGGACA	4620	
	K D N L T N L F R C M G Y T V I C K D N		
	270 280		
		intron 4	
4621	ATCTGACGGGAAGGGTACGGCGAAATTATATTACCCAAACGCGAAATTTGCCATTTTGCG	4680	
	L T G R		
		Repeat 3	
		----->	
4681	CCGAAAATGTGGCGCCCGGTCTCGACACGACAATTTGTGTTAAATGCAAAAATGTATAAT	4740	
	TTTGCAAAAAACAAAATTTTGAACCTCCGCGAAAATGATTTACCTAGTTTCGAAATTTTC	4800	
4741	GTITTTTCCGGCTACATTATGTGTTTTTCTTAGTTTTTCTATAATATTTGATGTAAAAA	4860	
4801	ACCGTTTGTAATTTTCAGACAATTTCCGCATACAAAACCTGATAGCAGGAAATCAATT	4920	
4861	TTCTGAATTTTCAAAATTATCCAAAATGCACAATTTAAATTTGTGAAATTTGCCAAAC	4980	
4921	GGTGTTCATATGAAATGTATTTTAAAAAAGTTTAAAAACCACTCCGGAAAAAGCAATAA	5040	
4981	AAATCAAAACAACGTCACAATTCAAATTCAAAAGTTATTTCATCCGATTTGTTTATTTTG	5100	
5041	CAAAATTTGAAAAATCATGAAGGATTTAGAAAAGTTTTATAACATTTTCTAGATTTT	5160	
5101	TCAAAATTTTAAAAAATCGAGAAAAAGAGAATGAAAAATCGAATTTAAAAATATCC	5220	
5161			
		Repeat 3	
		<-----	
5221	ACAGCTTCGAGAGTTTGAAATTACAGTACTCCTTAAAGGCGCACACCCCATTTGCATTGG	5280	
		-----	
5281	ACCAAAAATTTGTCGTGTCGAGACCAGGTACCGTAGTTTTTGTGCGAAAAATTCACCAT	5340	
5341	TGGACAATAAACCTTCCTAATCACCAAAAAGTAAATGAAATCTTCGAAAAAGCCAAAAA	5400	

FIGURE 3(e)  
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```

5401  ATTCAAAAAAAAAAGTCGAATTTTCGATTTTTTTTTTGGTTTTTTGGTCCCAAAAACCAAAA 5460
-----+-----+-----+-----+-----+-----+-----+-----+
5461  AAATCAATTTTCTGCAAAATACCAAAAAGAAACCCGAAAAAATTTCCAGCCTTGTTCCT 5520
-----+-----+-----+-----+-----+-----+-----+-----+
5521  AATGTAAACTGATATTTAATTTCCAGGGAATGCTCCTGACAATTCGAGACTTTGCCAAAC 5580
-----+-----+-----+-----+-----+-----+-----+-----+
                                |
                                G M L L T I R D F A K H
                                290                      300

5581  ACGAATCACACGGAGATTCTGCGATACTCGTGATTCTATCACACGGAGAAGAGAATGTGA 5640
-----+-----+-----+-----+-----+-----+-----+-----+
                                E S H G D S A I L V I L S H G E E N V I
                                310                      320

5641  TTATTGGAGTTGATGATATACCGATTAGTACACACGAGATATATGATCTTCTCAACGCGG 5700
-----+-----+-----+-----+-----+-----+-----+-----+
                                I G V D D I P I S T H E I Y D L L N A A
                                330                      340

                                A (n2433)
                                | | intron 5
5701  CAAATGCTCCCGTCTGGCGAATAAGCCGAAAAATCGTTTTTGTGCAGGCTTGTGAGGCG 5760
-----+-----+-----+-----+-----+-----+-----+-----+
                                N A P R L A N K P K I V F V Q A C R G E
                                350                      360

5761  GTTCGTTTTTTATTTTAATTTTAATATAAAATATTTTAAATAAATTCATTTTCAGAACGTC 5820
-----+-----+-----+-----+-----+-----+-----+-----+
                                                                R R

5821  GTGACAATGGATTCCCAGTCTTGGATTCTGTGACGGAGTTCCTGCATTTCTTCGTCGTG 5880
-----+-----+-----+-----+-----+-----+-----+-----+
                                D N G F P V L D S V D G V P A F L R R G
                                370                      380

                                T (n1165)
                                |
5881  GATGGGACAATCGAGACGGGCCATTGTTCAATTTTCTTGGATGTGTGCGGCCGCAAGTTC 5940
-----+-----+-----+-----+-----+-----+-----+-----+
                                W D N R D G P L F N F L G C V R P Q V Q
                                390                      400

| intron 6
5941  AGGTTGCAATTTAATTTCTTGAATGAGAATATTCCTTCAAAAAATCTAAAATAGATTTTT 6000
-----+-----+-----+-----+-----+-----+-----+-----+
6001  ATTCCAGAAAGTCCCGATCGAAAAATTGCGATATAATTACGAAATTTGTGATAAAATGAC 6060
-----+-----+-----+-----+-----+-----+-----+-----+

Repeat 4
6061  AAACCAATCAGCATCGTCGATCTCCGCCCACTTCATCGGATTGGTTTGAAAGTGGGCGGA 6120
-----+-----+-----+-----+-----+-----+-----+-----+

----->
6121  GTGAATTGCTGATTGGTCGCAGTTTTTCAGTTTAGAGGGAATTTAAAAATCGCCTTTTCGA 6180
-----+-----+-----+-----+-----+-----+-----+-----+
6181  AAATTAATAATTGATTTTTTCAATTTTTCGAAAAATATTCCGATTATTTTATATTCTTT 6240
-----+-----+-----+-----+-----+-----+-----+-----+

```

**FIGURE 3(f)**  
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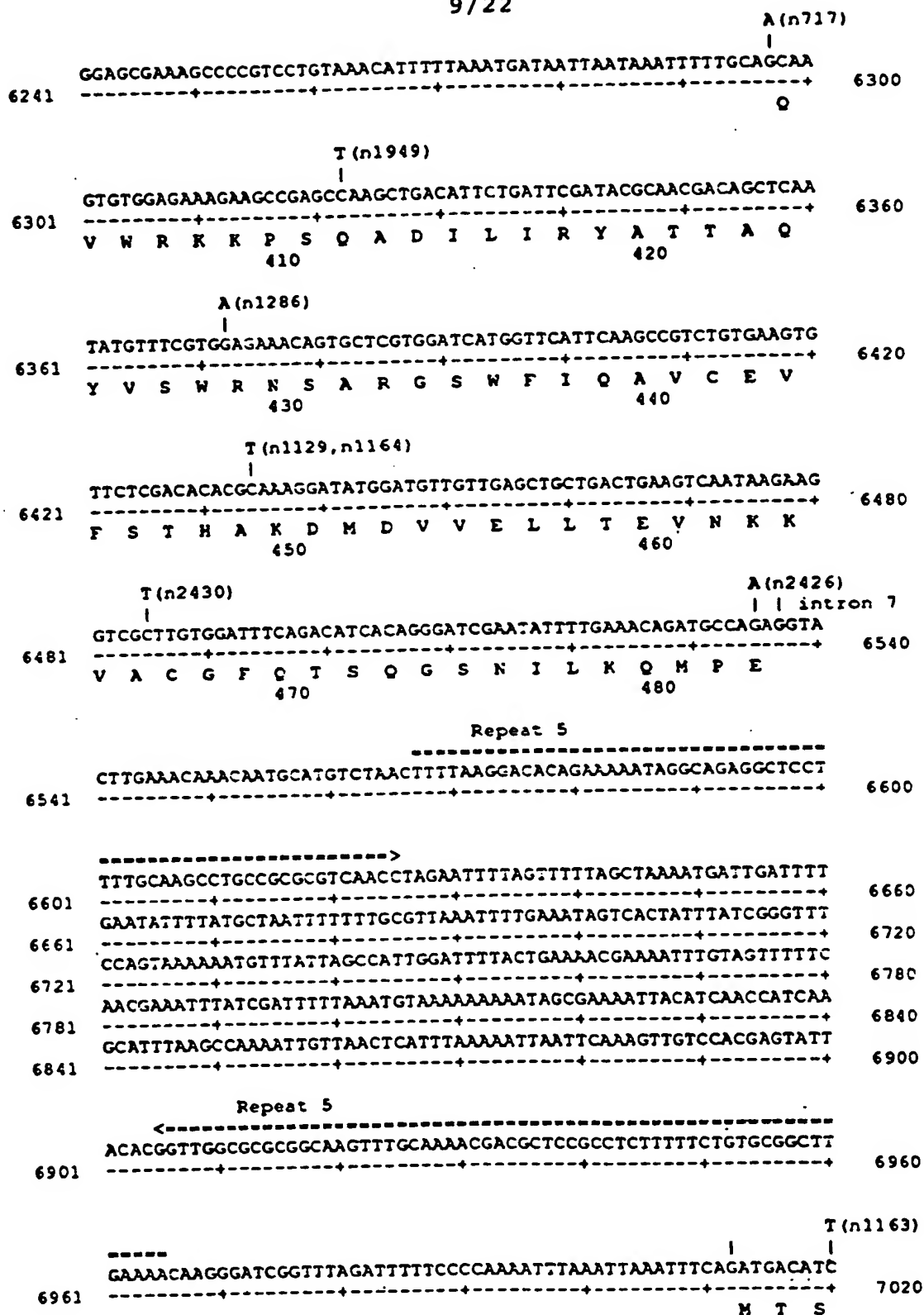


FIGURE 3(g)

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```
CCGCCTGCTCAAAAAGTTCTACTTTTGGCCGGAAGCACGAAACTCTGCCGTCTAAAATTC
-----+-----+-----+-----+-----+-----+-----+
7021 R L L K K F Y F W P E A R N S A V * 7080
      490                               500

ACTCGTGATTCAATTGCCCAATTGATAATTGTCTGTATCTTCTCCCCAGTTCTCTTTCGC
-----+-----+-----+-----+-----+-----+-----+
7081 CCAATTAGTTTAAAACCATGTGTATATTGTTATCCTATACTCATTTCACCTTATCATTCT 7140
-----+-----+-----+-----+-----+-----+-----+
7141 ATCATTCTCTTCCCATTTTCACACATTTCCATTTCTCTACGATAATCTAAAATTATGAC 7200
-----+-----+-----+-----+-----+-----+-----+
7201 GTTTGTGTCTCGAACGCATAATAATTTTAATAACTCGTTTTGAATTGATTAGTTGTTGT 7260
-----+-----+-----+-----+-----+-----+-----+
7261 GCCCAGTATATATGTATGTACTATGCTTCTATCAACAAAATAGTTTCATAGATCATCACC 7320
-----+-----+-----+-----+-----+-----+-----+
7321 CCAACCCCAACCAACCTACCGTACCATATTCATTTTGGCGGGAATCAATTCGATTAATT 7380
-----+-----+-----+-----+-----+-----+-----+
7381 TTAACCTATTTTTTCGCCACAAAAAATCTAATATTTGAATTAACGAATAGCATTCCCATC 7440
-----+-----+-----+-----+-----+-----+-----+
7441 TCTCCCGTGCCGGAATGCCTCCCGCCCTTTTAAAGTTCGGAACATTTGGCAATTATGTAT 7500
-----+-----+-----+-----+-----+-----+-----+
7501 AAATTTGTAGGTCCCCCCCATCATTCCCGCCCATCATCTCAAATTGCATTCTTTTTTCG 7560
-----+-----+-----+-----+-----+-----+-----+
7561 CCGTGATATCCCGATTCTGGTCAGCAAAGATCT 7620
7621 -----+-----+-----+-----+-----+-----+ 7653
```

FIGURE 3(h)

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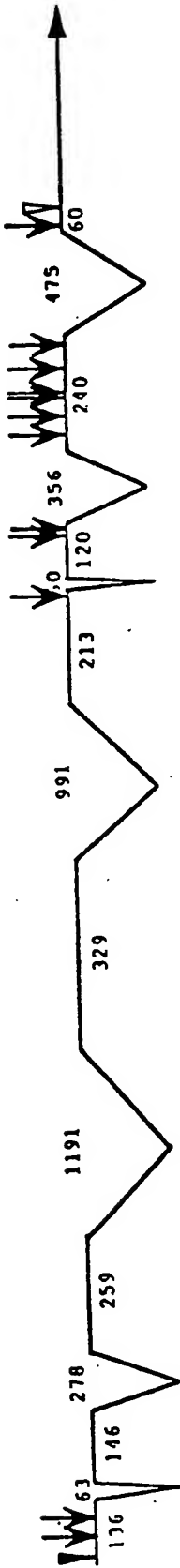


FIGURE 4(a)

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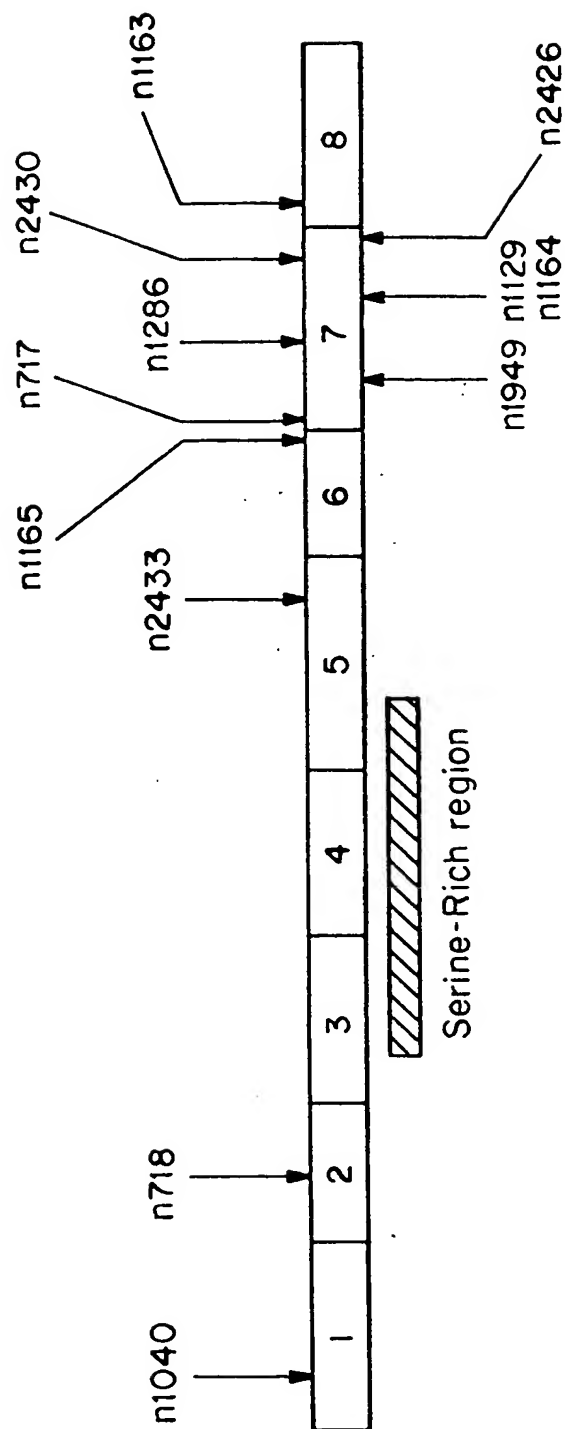


FIG. 4B

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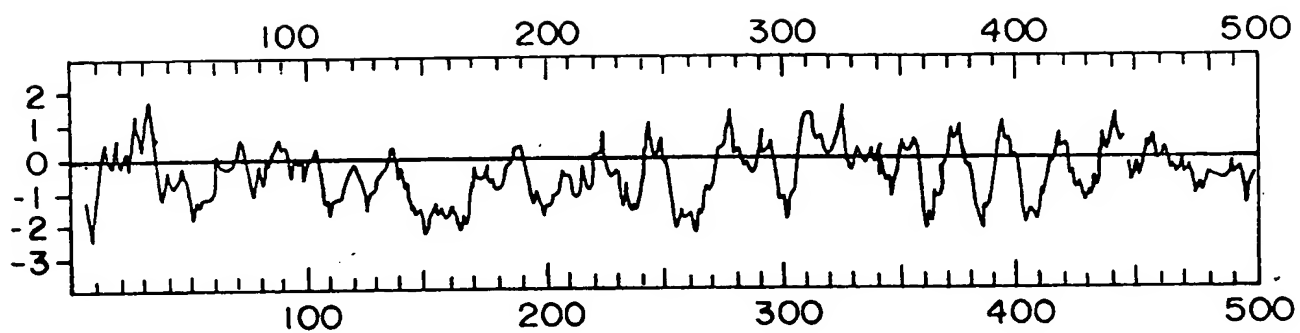


FIG. 5



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```

ICE      167  IICNEEFDSIPRRTGAEVDITGMTMLLQNLGYSVDVKKNLTASDMTTELE
           || |||. : :| |. |...| ..:| |:. :|||. | .|. ||| : : :| . :
Ced-3    247  IINNEHFQMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIR
BGAFQ     =====

217  AFAHRPEHKTSdstflvfmshgiregicgkKHSEQVPDI.LQLNAIFNML
     .|||. : :| |. |...| ..:| |:. :|||. | .|. ||| : : :| . :
297  DFAKHESH..GDSAILVILSHGEENVIIG.....VDDIPISTHEIYDLL
BGAFQ     =====

           active site      autocleavage site
266  NTKNCPSLKDKPKVIIIQACRGDSPGVVW.FKDSVGVSGNLSLPTTEEFE
     |. |. |. | : ||| : : : ||| | : : : . : . ||| : . : : . : : :
339  NAANAPRLANKPKIVFVQACRGERRDNGFPVLDSDGVPAFLRRGWDNRD
           ↓
           S
BGAFQ     =====

315  DDAI.....KKAHIEKDFIAFCSTPDNVSWRHPTMGSVFI
     :. : : :| . : | : : : : : : ||| : : : |||
389  GPLFNFLGCVRPQVQVWRKKPSQADILIRYATTAQYVSWRNSARGSWFI
           ↓                ↓
           stop              stop

351  GRLIEHMQEYACSCDVEEIF....RKVRFSFEQPDGRAQMPTT.ERVT.L
     . :. | : . : | . || | : : : :| : : : :| . : . : | . : |
439  QAVCEVFSTHAKDMDVVELLTEVNKKVACGFQTSQGSNILKQMPMTSRL
           ↓                ↓                ↓   ↓
           V                V                K   F

395  TRCFYLFPGH*..... 404
     : || : :| :
489  LKKFYFWPEARNSAV 503

```

FIGURE 6(b)

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```

251 EHFEQMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIRDFAK 300
      1 .....MLTVQVYRT 9
301 HESHGDSAILVILSHGEENVIIIGVDDIPISTHEIYDLLNAANAPRLANKP 350
      10 SQKCSSSKHV.....EVLLD....PLGT.SFCSLL.....PP 37
351 KIVFVQACRGERRDNGFPVLDSVDGVP AFLRRGWDNRDGP LFNFLGCVRP 400
      38 PLLLYETDRGVDQQDGKNHTQSPGC.....EESDAGKEELM..... 73
401 QVQCVWRKKPSQADILIRYATTAQYVSWRNSARGSWFIQAVCEVFSTHAK 450
      74 .....KMRLPTRSDMICGYACLKGNAAMRNTKRGSWYIEALTQVFSERAC 118
451 DMDVVELLTEVNK..KVACGFQTSQGSNIIKOMPENTSRL LKKFYFWPEA 498
      119 DMHVADMLVKVNALIKEREGYAPGTEFHRCKEMSEYCSITLCQQLYLFPGY 168
499 RNSAV 503
169 PPT*. 172

```

**FIGURE 7**

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## Alignment of N-terminal regions of ced-3/ICE-related proteins

C. briggsae ced-3 ced-3 protein	MMQDRWSLLERNILEFSSKIQADLILQVLIAKQVLNSDNGDVINSCHTERDNEKEIVKAVQRRGDEAFDAFYDALRDTGINDLADVLMPISR---PNPV	100
C. vulgaris ced-3 Mouse ICE.gw	MMQDRWSLLERNIMFSSHLKVDEILEVLIAKQVLNSDNGDMINSCGTVREKREIVKAVQRPQDVAFDAFYDALRSTGHEGLAEVLEPLARSVDNAV	100
Human ICE.GW	M-----ADKIL-----RAKRPQFINSV---SIGTINGLLDELLEK-----RVLNQEEM-----DKI	
Consensus	M-----ADKVL-----KEKRKLFIISM---GEGTINGLLDELLOQT-----RVLNKEEM-----EKV	
	M-----AD.IL-----R.KRK.....V---G-----D.L..T-----VL.....V	
C. briggsae ced-3 ced-3 protein	PMECMSPSSHRRSRALSPGYASPTRVHRDSISSVSSFTSTYQDVYSRABSSSRPLQSSDRHNMYGAA--TSFSPSPSBANSS--TGCCASLGYSSSRN	198
C. vulgaris ced-3 Mouse ICE.gw	EFECMSPASHRRSRALSPAGYTSPTRVHRDSVSSVSSFTSYQD--IYSRABSSRS--RALHSSDRHNYSPPVNAFPSPSSANSS--TGCCSLOYSSSRN	198
Human ICE.GW	-----STSSSRPLHTSDRHNYSVPS--NSFSQSPASANSS--TGSSSLGYSSSRT	
Consensus	KLA---NITAMDKARDLCHVSKKGPQASQIFITYICNEDCYL-----AGILELQSAPE--TFVAT-----EDSKGCHPSSETKEEQNKED-G	
	KRE---NATVMDKTRALIDSVIPKGAQACICITYICEEDSYL-----ACTGLSADQTSQ--NYLNM-----QDSQVLSFPAPQAVQDNPAMP	
	..E-----RAL.....I.....SY-----S.SRS.R.L.SSDRHNYS..S...F.SQP.BANSS--TG..SLOYSSSR.	
C. briggsae ced-3 ced-3 protein	RSFRTKTAQSQYIFHEEDKNVVDAPTTIHRVDFDEKTMIRNFSSPRFLCLIIINEFQOMPTRGITKADKDNLTNIFRCGYVICKNLNLTREM..STIRSF	298
C. vulgaris ced-3 Mouse ICE.gw	RSFSKASGPTQYIFHEEDKNFVDAPTTISRVDDEKTMIRNFSSPRFLCLIIINEFQOMPTRGITKADKDNLTNLFRCGYVICKNLNLTREM..LTIRLF	298
Human ICE.GW	RSYSKASAHSQYIFHEEDKNVVDAPTTIHRVDFDEKTMIRNFSTPRFLCLIIINEFQOMPTRGITKPKDNISNLFRCGYVICKNLNLTREM..--TIRCF	
Consensus	TFPGLTQTLKFCPLEKAOKLWKENPS--EIV--PIMNTT--TRTR--LALIIINEFPHLSFR/GHQVDLREMKLLLEDLOYVVKAKNLTLLEFVKEVKEF	
	TSSGSEQNVKLCSEEAQRILWKQKSA--EIV--PIMDKS--SRTR--LALIIINEFDSIPRRIGNEVDITGTMHLLQNLQLOYVVKAKNLTLSCMTTELEAF	
	RS.SK.B...OYIFHEEDKN.VDAPTI.RVDFDEKTMIRNF8.FBFLCLIIINEFQOMPTLGITK.DKDN.TNLFRCGYVICKNLNLTDR..M..TIR.F	
C. briggsae ced-3 ced-3 protein	GRNDMFI--GDSNIVLISGGENVITIG---VDDVS---VNVHEI--VLLNANAPILANKPKVVFVQACRG	360
C. vulgaris ced-3 Mouse ICE.gw	AKHESH--GDSNIVLISGGENVITIG---VDDIP---ISTHEI--VLLNANAPILANKPKVVFVQACRG	360
Human ICE.GW	AKNETH--GDSNIVLISGGENVITIG---VDDVS---VNVHEI--VLLNANAPILANKPKVVFVQACRG	
Consensus	AACPEHTSDSTFLVFSHGLOEGITIGTYSNEVSDILKVDTH--POMNTLKPILCKPK/IIDACRG	
	AHRPEHTSDSTFLVFSHGLOEGITIGKKHSEQVPDILQNALFMNLNFKNCPILCKPK/IIDACRG	
	A....H--GDSNIVLISGGENVITIG---VDDVS---VHEI--VLLNANAPILANKPKVVFVQACRG	

FIGURE 8(a)

### Alignment of the C-terminal regions of ced-3/ICE/NEDD-2 - related proteins

ICE C-terminus	DSPGVW---	---	FKDSVG-	---	-----V
Mouse ICE C-ter	EKGQWL---	---	LKDSVR-	---	-----D
C. briggsae C-ter.	ERRDNGFP--	---	VLDSDVG-	---	---RRGWDN
ced-3 C-terminus	ERRDNGFP--	---	VLDSDVG-	---	---RRGWDN
C. vulgaris C-terminus	ERRDVGFP--	---	VLDSDVG-	---	---RRGWDK
nedd-2 protein.gw	MLTVQVYRTS	QKCSSKHVV	EVLLDPLGTS	FCSLPPPPLL	LYETDRGVQDQ
Consensus	E.....	-----	LDsv...	-----P...	-----RG.D.

ICE C-terminus  
 Mouse ICE C-ter  
 C.briggsae C-ter  
 ced-3 Cterminus  
 C. vulgaris C-terminus  
 nedd-2 protein.gw  
 Consensus

SGNL---SLP TTEEF~~E~~---D DAIKKA-HIE KDFIAFCSST PDNVSWRHPT  
 SEE----DFL TDAIFE---D DGIKKA-HIE KDFIAFCSST PDNVSWRHPT  
 RDG-PLFNFL GCVRPQV--Q QVWRKK-PSQ ADMLIAYATT AQYVSWRNA  
 RDG-PLFNFL GCVRPQV--Q QVWRKK-PSQ ADILIRYATT AQYVSWRNA  
 GDG-P--NFL GCVRPQA--Q QVWRKK-PSQ ADILIAYATT AQYVSWRNA  
 QDGKHNHTQSP GCEESDAGKE ELMKMRLLPTR SDMICGYACL KGNAAMRNTK  
 .DG-....FL GC.....-.....K.-P.. D.....YA.T .VSWRN..

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ICE C-terminus	MGSLFI	GRGLI	EHMQEY	SC	DVERIFRKV	-RF----	SFE	QPDGRAQMPT	
Mouse ICE C-ter	RGS	FI	ESLI	KHMKEY	WSC	DLEDIFRKV	-RF---- <td>SFE</td> <td>QPEFRLQMPT</td>	SFE	QPEFRLQMPT
C. briggsae C-ter	RGS	FI	DAVC	EVFSLH	KDM	DVVELLTENV	KKVA--	CGFQ	TSQGSNILKQ
ced-3 Cterminus	RGS	FI	DAVC	EVFSTH	KDM	DVVELLTENV	KKVA--	CGFQ	TSQGSNILKQ
C. vulgaris C-terminus	RGS	FI	DAVC	EVFSLH	KDM	DVVELLTENV	KKVA--	CGFQ	TSQGANILKQ
nedd-2 protein.gw	RGS	NYI	EALT	QVFSER	ADM	IIVADMLVKVN	ALIKEREGVA	PGTEFHRCKE	
Consensus	RGS	NFIL	A..	EVFS..	A..	DM	DV..E..L..VN	.....-CGF..	.G....K.

ICE C-terminus	T_ERVT-LTR	CFVLPFH--	----
Mouse ICE C-ter	A_DRVT-LTK	RFVLPFH--	----
C.briggsae C-ter	MPELTSRLK	KFYFWPEDRG	RNSAV
ced-3 Cterminus	MPEMTSRLK	KFYFWPEAR-	-NSAV
C. vulgaris C-terminus	MPELTSRLK	KFYFWPEDRN	RSSAV
nedd-2 protein.gw	MSEYCSLTCC	QLYLPFG--	-YPPT
Consensus	M.E.TS.L.K	F.V.L.P....	-----

**FIGURE 8(b)**

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Lines

```

1 01 MMRQDRRSLLERNIMFSSHLKVDEELEVLIKQVLNSDNGDMINSCGTV 50
2 .....W_.....LE...K.QA.L..D.....V....R.E
3 TVSISLJ..R.....M.....

1 51 REKRREIVKAVQRPQDVAFDAFYDALRSTGHEGLAEVLEPLARSVDSNAV 100
2 .DNEK.....R..E.....D...ND..D..M..S.P .P.
3

1 101 EFECPMSPASHRRSRALSPAGYTSPTRVHESVSSVSFTS_YQDIYSRA 149
2 PM.....S.....P .A.....I.....T...V....
3 S

1 150 RSRSR_SRALHSSDRHNYSSPPVNAFP SQPSSANSSTGCSSLGYSRRN 198
2 ..S..S..P.Q.....M.AA_TS.....A.....
3 T...__..P..T.....V..S..S.Q...A.....S.....T

1 199 RSFSKASGPTCYIFHEEDMNFVDAPTI SRVFDEKTMYRNFSSPRGMCLI 247
2 .....T.AQS.....Y.....H.....L...
3 ..Y....AHS.....Y.....H.....T...L...

1 248 INNEHFECMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLTIRD 297
2 .....I.....E..S...S
3 .....P.....IS.....I.H.....M.....

1 298 FAKHESHGDSAILVILSHGEENVITGVDDIPITHEIYDLLNAANAPRLA 347
2 .GRNDM.....VSVTV.....
3 ...N.T.....VSVTV....X.....

1 348 NKPKIVFVQACRGERDNGFPVLES.DGVPAFLRGWDRDGPFLNFLGC 397
2 ....L.....SLI.....
3 ....L.....V.....LI.....KG...

1 398 VRPQVQQVWRKKPSQADILIRYATTACQYVSWRNSARGSWFIQAVCEVFST 447
2 .....M..A.....L
3 ....A.....A.....L

1 448 HAKDMDVVELLTEVNKKVACGFQTSQGSNLIKQMPENTSRLLKKFYFWPE 497
2 .....L.....
3 .....A.....L.....

1 498 _ARN__SAV 503
2 DRG..._...
3 _D..RS...

```

FIGURE 9

SUBSTITUTE SHEET

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Interleukin-1 $\beta$  convertase cDNA sequence

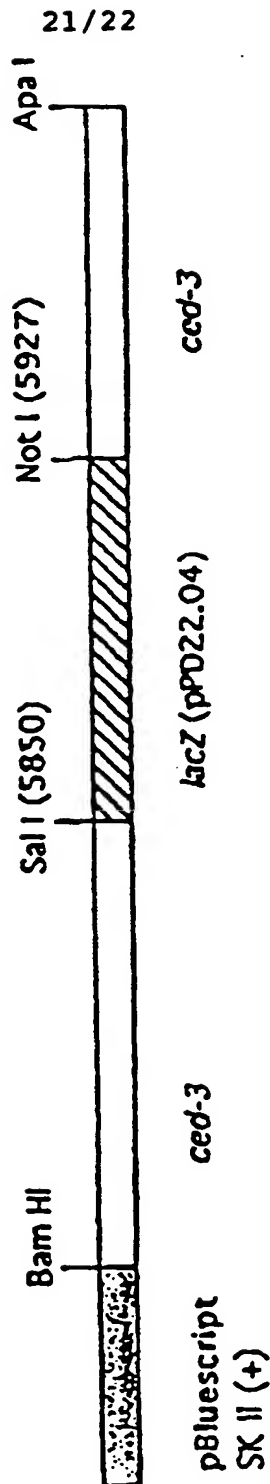
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1  AAAAGGAGAG AAAAGCCATG GCCGACAAGG TCCTGAAGGA GAAGAGAAAG
51  CTGTTTATCC GTTCCATGGG TGAAGGTACA ATAAATGGCT TACTGGATGA
101 ATTATTACAG ACAAGGGTGC TGAACAAGGA AGAGATGGAG AAAGTAAACG
151 GTGAAAATGC TACAGTTATG GATAAGACCC GAGCTTTGAT TGA CTCCGT
201 ATTCCGAAAG GGGCACAGGC ATGCCAAATT TGCATCACAT ACATTTGTGA
251 AGAAGACAGT TACCTGGCAG GGACGCTGGG ACTCTCAGCA GATCAAACAT
301 CTGGAAATTA CCTTAATATG CAAGACTCTC AAGGAGTACT TTCTTCCTTT
351 CCAGCTCCTC AGGCAGTGCA GGACAACCCA GCTATGCCCA CATCCTCAGG
401 CTCAGAAGGG AATGTCAAGC TTTGCTCCCT AGAAGAAGCT CAAAGGATAT
451 GGAAACAAAA GTCGGCAGAG ATTTATCCAA TAATGGACAA GTCAAGCCGC
501 ACACGTCTTG CTCTCATTAT CTGCAATGAA GAATTTGACA GTATTCCTAG
551 AAGAACTGGA GCTGAGGTTG ACATCACAGG CATGACAATG CTGCTACAAA
601 ATCTGGGGTA CAGCGTAGAT GTGAAAAAAT ATCTCACTGC TTCGGACATG
651 ACTACAGAGC TGGAGGCATT TGCACACCGC CCAGAGCACA AGACCTCTGA
701 CAGCACGTTT CTGGTGTTC TGTCTCATGG TATTCGGGAA GGCATTTGTG
751 GGAAGAAACA CTCTGAGCAA GTCCCAGATA TACTACAACT CAATGCAATC
801 TTAAACATGT TGAATACCAA GAAGTCCCA AGTTTGAAGG ACAAACCGAA
851 GGTGATCATC ATCCAGGCCT GCCGTGGTGA CAGCCCTGGT GTGGTGTGGT
901 TTAAAGATTG AGTAGGAGTT TCTGGAAACC TATCTTTACC AACTACAGAA
951 GAGTTTGAGG ATGATGCTAT TAAGAAAGCC CACATAGAGA AGGATTTTAT
1001 CGCTTTCTGC TCTCCACAC CAGATAATGT TTCTTGAGAG CATCCCACAA
1051 TGGGCTCTGT TTTTATTGGA AGACTCATTG AACATATGCA AGAATATGCC
1101 TGTTCTGTG ATGTGGAGGA AATTTTCCGC AAGGTTTCAT TTTCATTTGA
1151 GCAGCCAGAT GGTAGAGCGC AGATGCCAC CACTGAAAGA GTGACTTTGA
1201 CAAGATGTTT CTACCTCTTC CCAGGACATT AAAATAAGGA AACTGTATGA
1251 ATGTCTGCGG GCAGGAAGTG AAGAGATCGT TCTGTAAAAG GTTTTTGGAA
1301 TTATGTCTGC TGAATAATAA ACTTTTTTTG AAATAATAAA TCTGGTAGAA
1351 AAATGAAAAA AAAAAAAAAA AAA
```

FIGURE 10

SUBSTITUTE SHEET

# Constructs that Prevent Programmed Cell Death in *C. elegans*

BGAFQ construct



PBA construct

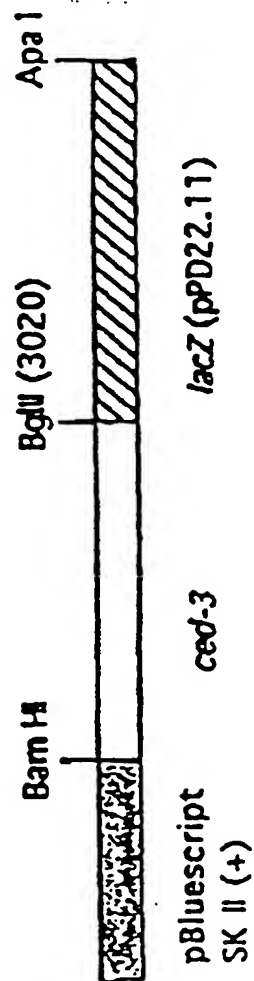


FIGURE 11(a)

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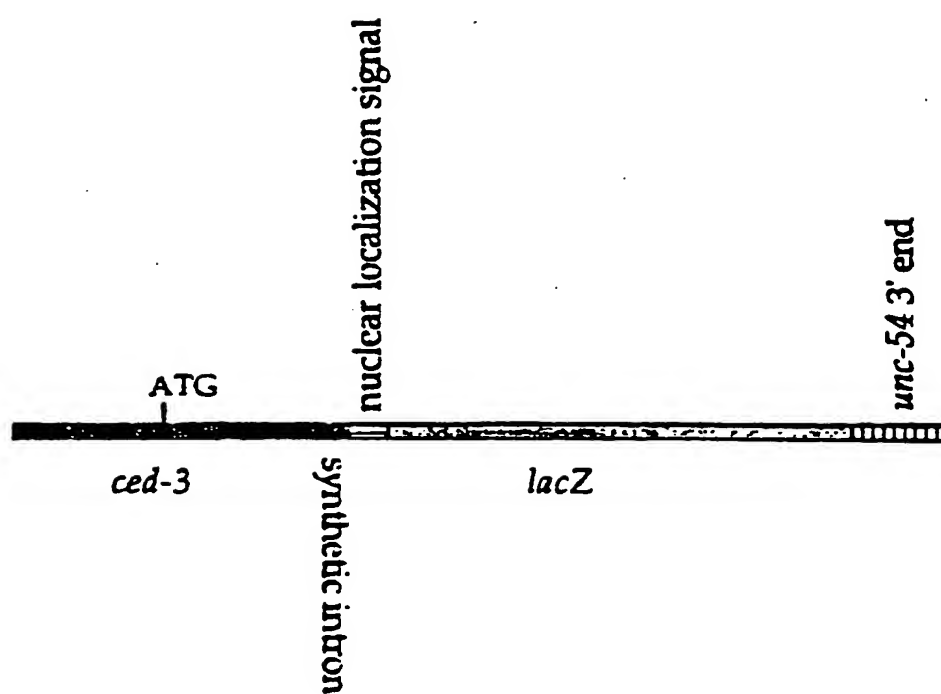


FIGURE 11(b)

SUBSTITUTE SHEET

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/05705

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/57; A61K37/02;	C12N9/64; C12Q1/68;	C12N15/39; G01N33/577; A61K31/70 G01N33/68
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ;      A61K ;      C12Q ;      G01N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	DISSERTATION ABSTRACT INTERNATIONAL B vol. 50, no. 10, April 1990, page 4431-B J. YUAN 'Genetic and molecular studies of ced-3 and ced-4: Two genes that control programmed cell death' see page 4431-B, left column, paragraph 2 abstract	1-13, 16-21, 28-45, 101-122
Y	WO,A,9 207 071 (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 30 April 1992 see page 6, line 1 - page 7, line 30; claims 1-27	1-45, 101-122
--- -/-		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
05 OCTOBER 1993	15 -10- 1993	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	HORNIG H.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 115 577 (IMMUNEX CORPORATION) 17 October 1991	54-56
Y	see page 3, line 25 - page 6, line 10; claims 1-30	14,15, 22-27, 101-122
	---	
X	NATURE vol. 356, 30 April 1992, MACMILLAN JOURNALS LTD., LONDON,UK; pages 768 - 774 N.A. THORNBERRY ET AL. 'A novel heterodimeric cystein protease is required for interleukin-1-beta processing in monocytes' cited in the application	54-57
Y	see page 770, right column, paragraph 2	14,15, 22-27, 101-122
	see page 773, right column, paragraph 2	
	---	
X	CELL vol. 69, no. 4, 15 May 1992, CELL PRESS, CAMBRIDGE, MA,US; pages 597 - 604 C.A. RAY ET AL. 'Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1-beta converting enzyme' cited in the application see page 599, right column, paragraph 2 - page 600, left column, paragraph 1	54,58
	---	
A	ANNUAL REVIEW OF CELL BIOLOGY vol. 7, 1991, ANNUAL REVIEW INC.,PALO ALTO,CA,US; pages 663 - 698 R.E. ELLIS ET AL. 'Mechanisms and functions of cell death' cited in the application the whole document	-
	---	
P,X	BIOCHEM. BIOPHYS. RES. COMMUN. vol. 185, no. 3, 30 June 1992, ACADEMIC PRESS, N.Y., US; pages 1155 - 1161 S. KUMAR ET AL. 'Identification of a set of genes with developmentally down-regulated expression in the mouse brain' cited in the application see page 1157, paragraph 4 - page 1161, paragraph 1; table 1	86,87
	---	
	-/--	



III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	<p>DATABASE WPI  Week 9318,  Derwent Publications Ltd., London, GB;  AN 93-144400  &amp; CA,A,2 076 159 (MERCK &amp; CO. INC.) 17  February 1993  see abstract</p> <p style="text-align: center;">---</p>	128
P,X	<p>EP,A,0 533 350 (MERCK &amp; CO. INC.)  24 March 1993</p> <p style="text-align: center;">---</p> <p>abstract</p>	<p>14, 15,  24,  42,  60, 128</p>
P,Y	<p style="text-align: center;">---</p> <p>NEUROBIOLOGY OF AGING  vol. 13, 1992, PERGAMON PRESS, NEW YORK,  US;  page S47  S. LEDOUX AND J. YUAN 'Isolation of  nematode homologs of the C. elegans cell  death gene ced-3'  Third international conference on  Alzheimer's disease and related disorders,  Abano terme, Italy, July 12-17, 1992;  Abstract no. 183;  abstract</p> <p style="text-align: center;">-----</p>	101-102

# INTERNATIONAL SEARCH REPORT

1. national application No.

PCT/US 93/05705

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark . Although claims 65,66,71,72 (as far as they concern in vivo treatment of human or animals) and claims 51,63,77,78 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9305705  
SA 76331

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 05/10/9

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9207071	30-04-92	US-A- 5217889	08-06-93
		AU-A- 9025091	20-05-92
		CA-A- 2094144	20-04-92
		EP-A- 0555370	18-08-93
WO-A-9115577	17-10-91	AU-A- 7775991	30-10-91
EP-A-0533350	24-03-93	None	